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F. J. BROWN
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VOLUME 43, NOS. 3, 4 NOVEMBER 1953

OTODISTOMUM PLUNKETI N.SP., A LARGE TREMATODE FROM A LORD PLUNKET'S SHARK, *SCYMNODON PLUNKETI* (WAITE)

BY M. L. FYFE, M.Sc.

University of Otago, New Zealand

(With 3 Figures in the Text)

INTRODUCTION

Thirty mature specimens of a large trematode were found in the body cavity of a Lord Plunket's shark *Scymnodon plunketi* (Waite) caught in Otago Harbour, N.Z. This is a southern New Zealand shark inhabiting water of 60–150 fathoms and is rarely taken by fishermen. The general arrangement of the organs and the relative size of the suckers, indicate that the trematode belongs to the genus *Otodistomum*, which was created by Stafford (1904) for trematodes found in the oesophagus, stomach and intestine of the skate, *Raja stabuliformis* (=*R. laevis*). These were identified as *Distoma veliporum* Creplin (1837) and renamed *Otodistomum veliporum* on account of their large size, without the new genus being defined. Lebour (1908) found a similar trematode in the stomach of the starry ray, and from it defined the genus *Otodistomum*. According to Dawes (1947, p. 214) the genus *Otodistomum* has only one species *O. veliporum* which is extremely variable and infects cartilaginous fish all over the world. One other possible species is *O. pristiophori* described by Johnston (1902) from three specimens found in the body cavity of the sawfish *Pristiophorus cirratus*. Odhner (1911) considered that Johnston's species was probably identical with *Otodistomum veliporum*, but Woolcock (1935) with one further specimen was able to extend Johnston's description and decided that this species should stand. Dawes, however, supports Odhner and makes *O. pristiophori* a probable synonym of *O. veliporum*.

O. veliporum has been recorded from New Zealand by Monticelli (1889) who lists without description *O. veliporum* (=*Distomum veliporum*) from the stomach of *Narcobatus* (=*Torpedo*) *fairchildi* and from the body cavity of *Raja nasuta*. No specimens of *Otodistomum* have been found in the body cavity of a large number of skates examined in the department of Zoology, and none were found in fourteen electric rays of the two species *Narcobatus fairchildi* and *Typhlonarke aysoni* examined by the author.

The characters of the trematode from the Lord Plunket's shark are sufficiently distinct from those of *Otodistomum veliporum* and *O. pristiophori* to warrant the formation of a new species.

DESCRIPTION (Fig. 1)

Body thick, broad and tapering to both ends (breadth not less than one-third of length). Large muscular parasites of this type can show a great range in body size and proportions depending on the degree of contraction. A series of sizes is given both for living and preserved specimens. Average size of living specimen, 65 by 33 mm.; of slightly flattened and preserved, 60–82 mm. by 33–27 mm.; of preserved without flattening, 46–62 mm. by 27–32 mm. Colour milky white with dark patches showing the position of eggs in uterus and of vitelline gland. Cuticle non-spinous, thick and wrinkled. Oral sucker 3·00–3·90 mm. in diameter; acetabulum 4·00–5·19 mm. in diameter; suckers ratio 1 : 1·5.

Pre-pharynx absent; pharynx conspicuous; oesophagus very short; caeca very long and undulating, reaching almost to the posterior end.

Genital pore median, nearer oral sucker than acetabulum, pore on raised papilla with thickened cuticle. Genital atrium narrow and deep (Fig. 2), size 2·5–3·3 mm. by 0·405–0·71 mm.; circular muscles surround atrium, longitudinal muscles connect with muscles of body wall, sphincter round entrance of atrium. Lining of atrium glandular and non-cuticular, except for outer edge where very thin cuticle extends in from surface of body. All specimens examined had ripe eggs in atrium, many of the eggs being imbedded in the glandular lining while indentations indicated the position where eggs had been.

Genital papilla at base of atrium, very small, non-muscular, non-cuticular, size 0·36–1·00 mm. by 0·19–0·33 mm. Of nine specimens sectioned none showed papilla everted. According to Manter (1926, p. 23) egg-laying occurs while the papilla is completely reduced, and in all these specimens egg-

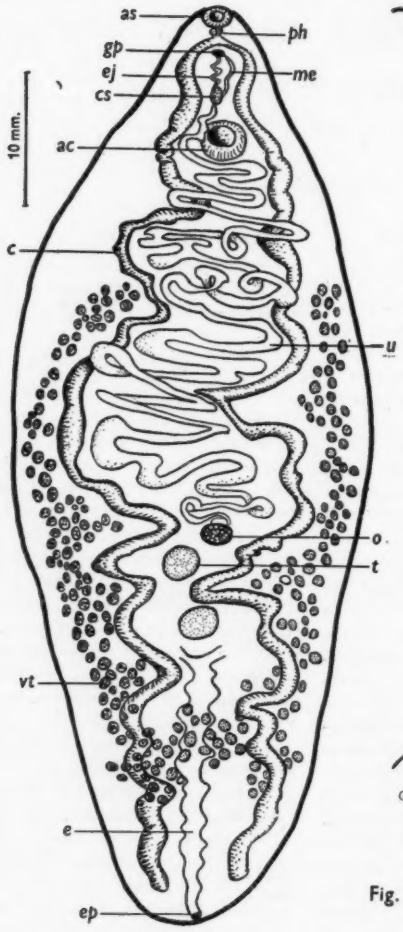

Fig. 1

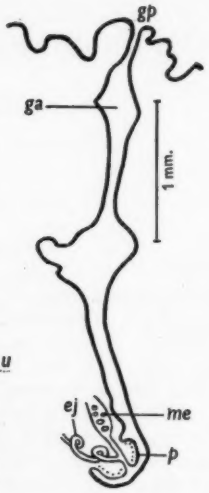

Fig. 2

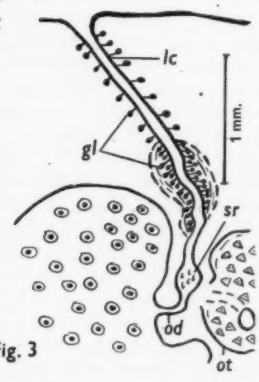

Fig. 3

Figs. 1–3. Fig. 1 was drawn by projection from a stained mounted specimen, Figs. 2 and 3 with the aid of a camera lucida. Fig. 1. *Otodistomum plunketi*. Dorsal view of adult seen by transparency. Scale = 10 mm. Fig. 2. Sagittal section through genital pore and atrium showing normal condition of genital papilla. Scale = 1 mm. Fig. 3. Transverse section through body showing Laurer's canal and oviduct entering seminal receptacle. Scale = 1 mm. *as*, anterior sucker; *ac*, acetabulum; *c*, caecum; *cs*, cirrus sac; *e*, excretory vesicle; *ep*, excretory pore; *ej*, ejaculatory duct; *ga*, genital atrium; *gl*, glands; *gp*, genital pore; *lc*, Laurer's canal; *me*, metraterm; *o*, ovary; *od*, oviduct; *ot*, ootype; *p*, genital papilla; *ph*, pharynx; *sr*, seminal receptacle; *t*, testis; *u*, uterus; *vt*, vitellaria.

laying was proceeding. Extrusion of papilla is probably brought about by contraction of body wall.

Cirrus sac between genital pore and acetabulum, large, ovoid, slightly muscular, size 1·60–1·35 mm., contains seminal vesicle and prostate gland; seminal vesicle wide, curved, tubular; prostatic duct leaves anterior end of seminal vesicle and turns back and then forwards receiving ducts of prostatic gland; narrow muscular ejaculatory duct connects prostatic duct with genital atrium.

Testes two, posterior to mid-body, rounded to elongate, tandem, average size of anterior testis 2·50 by 3·00 mm. Ovary in front and to the right of testes, average size 1·88 by 2·63 mm., flattened anteriorly and curved posteriorly. Testes and ovary not touching. Ootype anterior to ovary. Laurer's canal (Fig. 3) well developed, straight, leading slightly posteriorly, non-cuticular; longitudinal muscles and glands surround canal which becomes slightly convoluted and more glandular as it nears the ootype, the whole being surrounded by a muscular sheath; canal enters seminal receptacle at junction of oviduct and duct to uterus. Vitelline follicles in posterior two-thirds of body extending almost to posterior end. Uterus anterior to ovary; metraterm glandular and cuticular, joins ejaculatory duct as it enters genital papilla. Eggs thin-shelled, oval, average size (50 measurements) 0·111 by 0·075 mm., thickness of shell 0·009 mm. Excretory vesicle a long wide tube extending forward to level of posterior testis.

DISCUSSION

The characters of *O. plunketi* which are constant and distinct are listed below and are compared with those of *O. veliporum* and *O. pristiophori*, the latter being considered as a distinct species for the purpose of this discussion.

(1) *Position in the host.* *O. plunketi* and *O. pristiophori* are found in the body cavity, *O. veliporum* occurs in some part of the gut.

(2) *Size and shape.* The average size of thirty living *O. plunketi* is 65 × 33 mm., the breadth of both living and preserved specimens being never less than one-third of the length. Stafford (1904) gives the size of preserved *O. veliporum* as 30 by 3·5 mm., living specimens being up to 80 mm. long. If in the preserved contracted form the breadth is less than one-eighth of the length, in living specimens of twice the preserved length this ratio would be considerably lowered.

Lebour (1908) bases her generic diagnosis of *Otodistomum* on an animal measuring 32 by 2·6 mm., a much smaller trematode with breadth approximately one twelfth of the length.

Manter (1926) measured over 200 specimens of *O. veliporum* (= *O. cestoides*) varying in length from 2·3 to 65 mm. and found that the increase in width was very small compared with the increase in length. The youngest and shortest individuals were characteristically trematode-shaped with breadth approximately one-fifth of the length while the elongate cestode-like form was gradually assumed with age, the breadth of adults being one-twelfth of the length. It is evident from the above descriptions that the characteristic shape of *O. veliporum* is long and very narrow and that this shape is constant in all but very small, young specimens.

The average measurement of Johnston's three specimens of *O. pristiophori* was 25 by 6 mm., of Woolcock's one specimen, 14 by 5·5 mm. The number of specimens here measured is too small for any conclusion to be reached as to the range of size of this species. They are smaller than the average *O. veliporum*, but the ratio of breadth to length is similar to that of *O. veliporum* of the same size and, in accordance with Manter's finding, if they could extend to the length of *O. plunketi*, they would be very much narrower.

(3) *Gut caeca.* The caeca of *O. plunketi* follow a widely undulating course. The caeca of *O. pristiophori* are described as being 'thrown into bays and folds throughout their length' and in Johnston's figure (1902, pl. XIII, fig. 1) they have a slightly undulating appearance. In *O. veliporum* the caeca are straight.

(4) *Size of eggs.* The eggs of *O. plunketi* are much larger than those of *O. veliporum*, being 0·111 by 0·075 mm. as compared with 0·069 by 0·046 mm.

(5) *Ovary and testes.* The ovary and testes are well separated, the anterior testis being slightly larger than the posterior. In *O. veliporum* the ovary and testes touch, the anterior testis being smaller than the posterior. Some of these differences may be due to differences in contraction or fixation.

(6) *Seminal receptacle.* A seminal receptacle is present in *O. plunketi*, absent in *O. veliporum*.

REMARKS

The specific characters of *O. plunketi* are: large size and constant trematode shape, situation in the body cavity of its host, undulating caeca, large size of eggs. These characters are constant and are distinct from those of *O. veliporum*. The two accounts of *O. pristiophori* are based on very few specimens (four altogether). *O. pristiophori* is similar to *O. plunketi* in living in the body cavity of its host, but its very small size (smaller than the typical *O. veliporum*) and its elongate shape make it distinct from *O. plunketi*.

Special problems of taxonomy arise in the Trematoda because of host specificity and the difficulty of obtaining sufficient material. This was especially found in *O. plunketi* where the host fish is rarely caught, and related fishes which are known

to have contained *Otodistomum* appear to be rarely infected. It is realized that the defining of a species on the characters of a single population can possibly create taxonomic difficulties in that the material obtained may be the result of chance infestation. While the differences between *O. plunketi* and *O. veliporum* are mainly those of size and shape, it is not impossible that a closer relationship (i.e. subspecific) may exist.

SUMMARY

A new species, *Otodistomum plunketi*, is described from the Lord Plunket's Shark *Scymnodon plunketi* caught in Otago Harbour, New Zealand. This species is compared with *Otodistomum veliporum* and *O. pristiophori*, from which it is mainly distinguished by its size, shape, egg-size and undulating caeca.

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A NOTE ON THE ELECTRON MICROSCOPY OF THE TURNIP YELLOW MOSAIC VIRUS

KENNETH M. SMITH, *Agricultural Research Council, Plant Virus Research Unit, Molteno Institute, Cambridge*

(With Plate IX)

The recently discovered virus of turnip yellow mosaic (Markham & Kenneth Smith, 1949) has several interesting properties. It is transmitted in nature by a biting insect, a flea beetle, instead of the more usual sap-sucking insect. It is also the first insect-transmitted virus to be crystallized, and it can easily be isolated from the plant and purified to form octahedral crystals. The most interesting phenomenon, however, is the invariable occurrence in the infected plant of two components of the virus. If a purified solution of the virus is spun on a Sorval centrifuge for 2 hr. at about 12,000 r.p.m., it separates into two portions with a sharply defined boundary. These portions consist of a top component and a mixture of the top and a bottom component. Considerable further fractionation is required to get the bottom component free of the top. When separated the top and bottom components are apparently identical, except that the top component contains no nucleic acid and does not multiply in the plant.

Markham (1951) has made a physicochemical study of the two proteins isolated from plants diseased with turnip yellow mosaic. From the properties of the two particles there seems little doubt that, as far as their surfaces are concerned, the top and bottom components are extremely similar if not identical. The particles form mixed crystals in a lattice which calls for unusual specificity. Bernal & Carlisle (1948) have shown that the normal crystals are of the diamond type, a type which requires a high degree of symmetry of the particle surfaces. Furthermore, the inference is that the nucleic acid in the virus, or bottom component, is in the centre, either as a solid mass or, more probably, forming a sponge-like structure which in the top non-infectious component is occupied by water. It therefore seemed worth while to make a study of the two types of particles under the electron microscope to observe any difference in the behaviour of the particles under these conditions. It seemed possible that if the particles of the top component were more or less hollow spheres, containing water at the centre, then the effect of drying *in vacuo* inside the electron microscope might cause the particles to collapse or flatten somewhat. Under these circumstances they should throw a shorter shadow than the apparently more solid particles of the bottom, or virus, component. Dr Markham kindly supplied the writer with separate purified solutions of the top and bottom components. Samples of each were heavily diluted so that only a comparatively few particles would appear, more or less evenly distributed in the field of the microscope.

Drops of each were then placed on formvar-covered grids and shadowed with palladium. Great care was taken that the two samples should receive exactly the same treatment and they were shadowed together at the same time and at the same angle. They were then photographed on an R.C.A. electron microscope at a magnification of 20,000. A series of photographs was taken and the shadows thrown by the respective types of particle were carefully measured. No significant difference, however, could be detected in the length of the shadow thrown. It is interesting to find that the apparently hollow particles still retain their spherical shape under the conditions of drying in the microscope.

Pl. IX, fig. 1 is a photograph of a highly diluted preparation of the top component, whilst fig. 2 is a similarly treated solution of the bottom component. It will be seen that the respective particles in figs. 1 and 2 are apparently identical in size and appearance. In some instances the particles are aggregating in pairs. Both photographs were taken at a magnification on the microscope of $\times 20{,}000$.

Some attempts were next made to examine the virus within the cells of the infected plant. The only way to do this on the electron microscope is by means of very thin sections. For this purpose young infected plants of Chinese Cabbage (*Brassica chinensis*) showing good symptoms were used. Small pieces were cut out of the mottled leaves and fixed in osmic acid. They were then embedded in methacrylate and sectioned on a rotary-type microtome using the method of thermal expansion. The plastic was removed with amyl acetate and the sections were shadowed with palladium and photographed on the R.C.A. electron microscope. Examination of the diseased cells revealed the presence of large numbers of spherical particles of

uniform size scattered throughout the cells. They were not observed in normal cells. The size and appearance of these particles are similar to those of the turnip yellow mosaic virus, and it is concluded that they probably are the virus particles. It is noteworthy that in the sections examined the number of particles seemed considerably fewer than in corresponding sections of *Datura* leaves infected with the virus of tomato bushy stunt (Smith, 1953). The general appearance of the infected tissue is shown in Pl. IX, fig. 3, which is a section through a whole cell and part of another, photographed at a low magnification (× 4000). In fig. 4 is another section of infected tissue photographed at a higher magnification (× 8000). Numbers of uniformly sized particles, which are considered to be virus particles, can be seen. The opaque white bodies of different sizes in the centre of the photograph are probably salt crystals.

SUMMARY

An attempt to differentiate by means of the electron microscope between particles of the top and bottom components of the turnip yellow mosaic virus is described. Since the particles of the top component are thought to be hollow spheres, it might be expected that they would collapse after drying and thus throw a smaller shadow than the particles of the bottom component which are thought to have a more solid centre. No difference could be perceived between the two types of particle, so that the apparently hollow particles can withstand the effect of drying without collapsing.

Sections were cut of leaves of Chinese cabbage (*Brassica chinensis*) and particles were observed inside the cells which could conceivably be virus particles.

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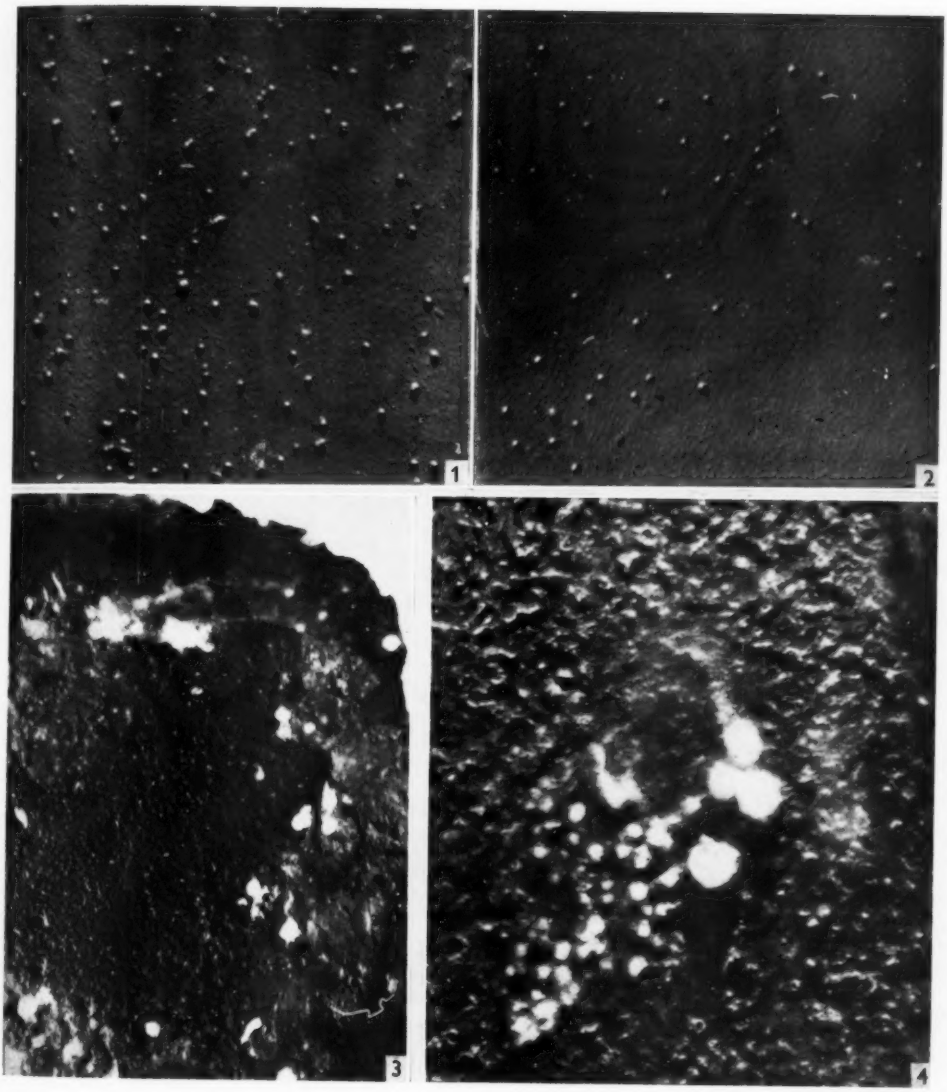
EXPLANATION OF PLATE IX

Fig. 1. Top component of the turnip yellow mosaic virus, highly diluted. × 16,000.

Fig. 2. Bottom component of the turnip yellow mosaic virus, highly diluted. × 16,000

Fig. 3. Thin section through part of a leaf of Chinese cabbage (*Brassica chinensis*) infected with the turnip yellow mosaic virus. × 4000

Fig. 4. Thin section through part of a cell of a leaf of Chinese cabbage, infected with the turnip yellow mosaic virus; note the numerous uniform particles which may be virus particles. × 8000.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SOME PARASITIC WORMS FROM FISHES OFF THE COAST OF ICELAND

III. MONOGENEA, NEMATODA, ACANTHOCEPHALA

By GWENDOLEN REES, D.Sc.

Department of Zoology, University College of Wales, Aberystwyth

The present paper is the third of a series dealing with a collection of parasitic worms from fishes off the coast of Iceland. The two previous papers have been concerned with the Cestoda and Digenea respectively (Rees, 1953*a*, *b*). There are no existing records of Monogenea, Nematoda or Acanthocephala from fishes off the coast of Iceland. Although this collection is not a large one, it indicates a wider geographical range of parasites already recorded from marine fishes in other parts of the world. There are five new host records.

MONOGENEA

The monogenetic trematodes of fishes have been studied, mainly, in the northern hemisphere off the coasts of Europe, North America, Canada and Japan. Very little is known of forms occurring on fishes off the coasts of South America, Africa or in the Antarctic. Most attention has been paid to fishes from coastal waters; those from the deep sea have been less frequently examined.

Nicoll (1915) compiled a list of all the British marine fishes from which trematodes had been recorded in Britain, together with a supplementary list of parasites which had been reported from British fishes outside British waters. This included sixty-five species of Monogenea. Nicoll incorporated into his list details from various British surveys, namely, from Scottish waters by T. Scott, from Irish waters by A. Scott, Bellingham and Southern, and from the Northumberland coast by Lebour. Records for British fish outside British waters he obtained from the works of Rudolphi, Monticelli, Stossich and Looss in southern Europe and from those of Odhner, Olsson and Cerfontaine in more northerly regions.

Since the publication of Nicoll's list surveys have continued around the British Isles. Little (1929) examined fishes from the west of Ireland, Baylis & Jones (1933) from Plymouth and Rees & Llewellyn (1941) from the Irish Atlantic Slope and Porcupine Bank. Outside British waters monogenetic parasites of marine fishes have been studied by various writers, among them Stafford (1904) and Cooper (1915) in Canada, Manter (1926), MacCallum (1932) and Price (1937, 1943) in North America, Olsson (1876) and Brinkmann (1940) in Scandinavia, Johnston & Tiegs (1922) in Australia and Goto (1894) and Yamaguti (1934–6) in Japan.

The more recent monographs by Sproston (1946) and Dawes (1947) are most valuable to anyone working in this field.

In the present instance three species of Monogenea were found. A classified list of them is given in Table 1 followed by a brief account of each, together with details of the exact locality and depth from which the host fishes were taken.

Table 1. *List of Monogenea with their fish hosts*

Suborder Monopisthocotylea.
  Superfam. Gyrodactyloidea.
    Fam. Dactylogyridae.
      Subfam. Tetraonchinae.
        (1) *Linguadactyla molvae* Brinkmann, 1940; *Molva byrkelange* Walbaum.

Suborder Polyopisthocotylea.
  Superfam. Diclidophoroidea.
    Fam. Diclidophoridae.
      (2) *Diclidophora denticulata* (Olsson, 1876); *Gadus virens* L.

  Superfam. Polystomatoidea.
    Fam. Rajonchocotylinae.
      (3) *Rajonchocotyloides emarginata* (Olsson, 1876); *Raja clavata* L.

(1) **Linguadactyla molvae** Brinkmann, 1940

Host. *Molva byrkelange* Walbaum.
Locality. East coast of Iceland, 15° 15′ W., 63° 47′ N.
Depth. 140 fathoms.
Incidence. 21 August 1948. 1 specimen.
Habitat. Gills.

Linguadactyla molvae was described by Brinkmann (1940) from the gills of the blue ling, *Molva byrkelange* Walbaum, in Norway, specimens being found on twelve of nineteen host fish examined. As far as the writer is aware there has been no other record of the species until now, when a single specimen has been recovered from the gills of a blue ling caught off the coast of Iceland. Brinkmann, in

addition to describing the worm, discussed its systematic position. He placed it in the family Gyrodactylidae Cobbold, 1864. Sproston (1946) placed the worm in the family Dactylogyridae Bychowsky, 1933 (Gyrodactylidae Cobbold, 1864, in part; Amphibdellidae Caries, 1885, in part), an arrangement which has been accepted by subsequent authors. Following the key of Johnston & Tiegs (1922) *Linguadactyla molvae* falls into the subfamily Tetraonchinae Monticelli, 1903. Dawes (1947) followed the same classification; he gave a diagnosis of the species and is of the opinion that it is 'likely to appear in Britain'.

The present specimen was removed from the host before it was given to the writer, but according to Brinkmann the worm is permanently attached to the gills of the host, the consequent irritation causing a ring-like growth of tissue around the opisthaptor. In all morphological features the specimen agrees with Brinkmann's description except that it is 7·44 mm. long instead of Brinkmann's measurement of 4–6 mm. The greatest breadth, of 2 mm., immediately behind the middle of the body is identical in both cases. The shape is elliptical and the body is slightly concave dorsally and ventrally. The vitellaria form prominent dark bands on either side of the yellowish white body. The dimensions of the two pairs of large hooks on the opisthaptor agree exactly with those given by Brinkmann, who also mentioned six (seven ?) small 'chitinous spines' in a semicircle on either side of the hooks. In the present specimen there are seven of these on either side. The mouth is subterminal and bordered by a large dorsal and a small ventral lip. The measurements of the pharynx are greater than those given by Brinkmann, being 0·64 × 0·53 mm. as compared with 0·45 × 0·28 mm. There is no oesophagus, and the branched caeca extend to the posterior third of the body. The arrangement of the reproductive organs agrees with that of the original description.

(2) **Diclidophora denticulata** (Olsson, 1876) Price, 1943

Synonymy: *Octobothrium denticulatum* Olsson, 1876; *Dactylocotyle denticulatum* (Olsson) of Cerfontaine, 1895; *D. carbonarii* Cerfontaine, 1895.

Diclidophora denticulata was first described by Olsson in 1876 as *Octobothrium denticulatum* on the gills of *Gadus virens* from the Skagerrak, five specimens being found on two of five fishes examined. Cerfontaine (1898) described the same worm as *Dactylocotyle denticulatum* and *D. carbonarii* on the gills of *Gadus carbonarius* from Ostend. The two generic names *Octobothrium* Leuckart, 1827 and *Dactylocotyle* Marshall, 1873 have been used for the species by various writers, the former by Linton (1899) and Little (1929) and the latter by Stafford (1904), Cooper (1915), Nicoll (1915), Sprehn (1933), Baylis & Jones (1933) and Rees & Llewellyn (1941). It was placed in the genus *Diclidophora* by Price (1943).

In Britain *Diclidophora denticulata* has been found at Aberdeen by Nicoll (1915), off the Irish coast by Little (1929), in the North Sea by Sprehn (1933), at Plymouth by Baylis & Jones (1933) and from the Irish Atlantic Slope by Rees & Llewellyn (1941). Other European localities from which it has been reported are Skagerrak by Olsson (1876), Ostend by Cerfontaine (1896) and Elba by Parona in 1899. Outside Europe it has been recorded at Woods Hole by Linton (1899), in Canadian waters by Stafford (1904) and from St Mas Bay, Nova Scotia, by Cooper (1915). The host in all cases except one was *Gadus virens*. Baylis & Jones (1933) describe the host as *Merluccius merluccius*, but Sproston (1946) suggested that this was a misidentification, as superficially the species resembles *Gadus virens*.

Diclidophora denticulata can be identified, mainly, by the form of the clamps which are borne on relatively stout peduncles. Their complicated structure has been described by Cerfontaine (1898), who indicated that their main feature is the presence on the external surface of the anterior or ventral valve of thirty to forty small spines. Olsson (1876) gave the length of the worm as 7 mm. and Cerfontaine (1898) as 6–12 mm. The present specimens measure 8–9 mm. The body tapers from the trapezoidal disk towards the anterior end. The male and female pores are separate and the penis bears four hooks. The appendages of the eggs are characteristic, one being short and curved and the other long and terminated by a crenulated swelling.

(3) **Rajonchocotyloides emarginata** (Olsson, 1876) Price, 1940

Synonymy: *Onchocotyle emarginata* Olsson, 1876; *O. appendiculata* Sonsino, 1890 nec. Kuhn, 1829.

Host	*Raja clavata* L.	
Locality	East coast of Iceland: 11° 14′ W., 65° 58′ N.	10° 54′ W., 65° 55′ N.
Depth	65 fathoms	160 fathoms
Incidence	19 August 1948, 4 specimens	20 August 1948, 10 specimens
Habitat	Gills	Gills

Rajonchocotyloides emarginata was first described by Olsson (1876) as *Onchocotyle emarginata*. The description was based on a single slightly damaged specimen on the gills of *Raja clavata* off the coast of Sweden. Sonsino described the species as *Onchocotyle appendiculata* and also recorded the same species as *O. emarginata* from *Raja clavata* off the Italian coast. Cerfontaine (1900) reported *Onchocotyle emarginata* from the same host species and included

a key for the identification of five species of the genus. Sprehn (1933) found it in the North Sea and Baylis & Jones (1933) recorded *O. appendiculata* on *Raja clavata* at Plymouth. Price (1940) redescribed the species from a single specimen, sent from the Plymouth collection of Baylis & Jones, as the type of a new genus *Rajonchocotyloides* and considered it identical with *Onchocotyle emarginata* Olsson. The erection of the new genus was based mainly on the presence of vitellaria in the appendix of the opisthaptor. Sproston (1946) collected specimens from Roscoff, Plymouth and Bangor which agree with Price's description and which she placed in the genus *Rajonchocotyle*. In the latter genus the vitellaria do not extend into the appendix of the opisthaptor. Dawes (1947) retained the generic name *Rajonchocotyloides*, but added a note that Price had chosen an inconstant character as 'In many Hexabothriids the vitelline follicles enter or fail to enter the appendage in different individuals of the same species'.

Sproston examined over five hundred specimens and found vitellaria in the appendix in each case. Dawes (1947) examined six at Plymouth, all of which contained vitellaria in the appendix, and this is true of the fourteen specimens which the writer has obtained off the coast of Iceland. There is no evidence of the presence of vitellaria in the appendix in the type species of the genus *Rajonchocotyle*; the generic name *Rajonchocotyloides*, therefore, is retained here.

So far the species has been recorded only in European waters off the coasts of Sweden, Britain, France and Italy.

The present specimens of *Rajonchocotyloides emarginata* resemble, in all anatomical details, descriptions given by previous authors, but there is some variation in recorded measurements. In the following brief description measurements taken from the fourteen specimens collected off the coast of Iceland are given together with those of previous authors, for comparison, in parentheses. The body is 6·5–7·7 mm. long with a maximum breadth of 1·72–1·93 mm. (Olsson (1876) 12 × 0·9 mm.; Price (1940) 5 × 1·8 mm.; Sproston (1946) up to 10·05 × 1·59 mm.; Dawes (1947) 2·5–4·2 × 0·55–1·10 mm.). The transversely oval oral sucker is 0·48 mm. in diameter (Olsson 0·4 mm.; Price 0·285 mm.; Sproston 0·375 mm.; Dawes 0·29–0·31 mm.) and the opisthaptor 2·68 mm. (Price 2·5 mm.; Dawes 1·35–1·75 mm.). The appendix is 2·35 mm. long and 0·64 mm. broad (Price 1·36 × 0·51 mm.; Dawes 1·15–1·20 × 0·28–0·48 mm.). The dimensions of the six suckers on the cotylophore and the two on the appendix show less variation. The diameter of the pharynx agrees fairly closely with that given by Price and exceeds slightly that given by Dawes. The very short oesophagus is followed by two intestinal limbs which give off diverticula and which unite in the cotylophore, sending a median branch into the appendix almost as far as the terminal suckers. In all the present specimens the vitellaria extend from the pharynx throughout the length of the body and are continued into the appendix, terminating a short distance before the extremity of the gut diverticulum. The eggs are very characteristic in shape, being described by Dawes as 'rugby-football shaped' and provided with meridional bands. They are 0·29 × 0·087 mm. (Price 0·17–0·197 × 0·08–0·097 mm.; Sproston 2·15 × 0·083 mm.; Dawes 0·183–0·219 × 0·067–0·086 mm.).

NEMATODA

Nematode parasites of fishes have, on the whole, received less attention than the trematodes and cestodes. Records around the coasts of the British Isles are rare. Baylis (1928, 1939) included some nematodes from fishes in his list of parasites of British vertebrates, and Baylis & Jones (1933) have mentioned nine species from fishes at Plymouth. The writer (1945, 1946) has recorded fourteen species in fishes from the Porcupine Bank, Irish Atlantic Slope and Irish Sea.

Records of fish nematodes have been made from time to time in other European waters by Rudolphi (1809), von Linstow (1899), Dujardin (1845),

Table 2. *List of nematodes with their fish hosts*

Order Ascaroidea	
Fam. Ascaridae	
Subfam. Anisakinae	
(1) *Contracaecum clavatum* (Rud.).	*Gadus virens* L.
	G. callarias L.
(2) *Contracaecum* sp., larva.	*Anarhichas minor* Olafsen.
(3) Anisakis or Porrocaecum, larva.	*Anarhichas minor* Olafsen.
	Gadus callarias L.
	Clupea harengus L.
Order Filarioidea	
Fam. Spiruridae	
(4) *Proleptus acutus* Duj.	*Raja clavata* L.

Diesing (1851), Wülker (1930) and others. Linton (1901) included a number in his account of parasites of fishes from Woods Hole, and Yamaguti (1934–6) dealt with thirty-seven species from fishes in Japanese waters.

No nematodes have as yet been recorded from fishes in Icelandic waters. In the present investigation four species were found and they are classified with their hosts in Table 2. Following this a brief account of each is given, together with details of the locality and depth in which the host fishes were found.

(1) **Contracaecum clavatum** (Rud. 1809)

Synonymy.	*Ascaris clavata* Rud.	*A. gadi* Muller
Hosts.	*Gadus virens* L.	*Gadus callarias* L.
Locality.	12° 1′ W., 66° 10′ N.	12° 7′ W., 66° 10′ N.
Depth.	58 fathoms	58 fathoms
Incidence.	18 August 1948, 9 females	18 August 1948, 2 females
Habitat.	Stomach	Stomach

Contracaecum clavatum was first described by Rudolphi (1809) from *Gadus barbatus* (†*Mullus barbatus* Day) and *G. aeglifinus*. It is a very common parasite of fishes and has been recorded around the British Isles by Taylor (1925), Baylis (1928), Baylis & Jones (1933) and Rees (1945, 1946) from a large number of host fishes, namely, *Gadus callarias* L., *G. virens* L., *G. merlangus* L., *G. aeglifinus* L., *G. pollachius* L., *Merluccius merluccius* (L.), *Hippoglossus hippoglossus* (L.), *Scomber scombrus* L., *Mullus surmuletus* L., *Lophius piscatorius* L., *Capros aper* (L.), *Cottus bubalis* Euphrasen, *C. scorpius* L., *Onos mustellus* (L.), *Conger conger* (L.), *Trachurus trachurus* (L.), *Belone belone* (L.), *Salmo trutta* L., *S. salar* L., *Solea* sp. and *Raja* sp.

It has been recorded from some of the same hosts in other European waters and at Woods Hole by Linton (1901) from *Scomberomorus maculatus*, *Pomolobus mediocris* and *Scomber scombrus*. Yamaguti does not record it from Japan.

The nearest locality to Iceland where *Contracaecum clavatum* has previously been found is the Irish Atlantic Slope where the writer (1946) reported it from *Gadus virens*, *G. merlangus*, *Trachurus trachurus* and *Scomber scombrus*.

Nine females were recovered from *Gadus virens* and two females from *G. callarias*. They varied in length from 67 to 82 mm. and in breadth from 0·73 to 1·10 mm. The species is adequately described.

(2) **Contracaecum** sp., larva

Host. *Anarhichas minor* Olafsen.
Locality. East coast of Iceland, 12° 33′ W, 66° 27′ N.
Depth. 65 fathoms.
Incidence. 19 August 1948, 2 specimens.
Habitat. Body cavity.

Larvae of various species of *Contracaecum* have been recorded from marine fishes. Yamaguti (1934–6) found larvae in twenty-three species of host fishes in Japan. As it is impossible to identify the species from the larval stage, Yamaguti divided them into five types, namely, (1) larvae with numerous terminal tail spines, (2) larvae with seven terminal tail spines, (3) larvae with a single very small tail spine, (4) larvae with boring tooth and moderately long ventricular appendix and (5) larvae with boring tooth and exceedingly long ventricular appendix. The present specimens fall into the third group, having a single very small tail spine. Yamaguti records this type from four hosts.

There is no previous record of a larva of *Contracaecum* from *Anarhichas minor* in which host two specimens have now been found.

(3) **Anisakis** or **Porrocaecum**, larva

Hosts.	*Anarhichas minor* Olafsen.	*Gadus callarias* L.	*Clupea harengus* L.
Locality.	12° 33′ W., 60° 27′ N.	12° 7′ W., 66° 16′ N.	11° 40′ W., 66° 5′ N.
Depth.	65 fathoms	68 fathoms	58 fathoms
Incidence.	19 August 1948, large number	19 August 1948, 1 specimen	19 August 1948, 4 specimens
Habitat.	Liver	Liver	Liver

Larval nematodes of this type have been recorded previously from a number of host fishes. They all possess an elongated ventriculus in the oesophagus, a character of adults of the genus *Anisakis*. They may, therefore, be the larval stages of a species of this genus or they may be larvae belonging to the genus *Porrocaecum*. In the latter case the larva has an oesophagus exactly similar to the adult stage of *Anisakis*, the intestinal caecum developing later. Baylis has suggested that young larval ascarids of this type cannot be identified beyond referring to them as *Anisakis* or *Porrocaecum* sp. It seems likely that the adult stage occurs in the alimentary canal of marine mammals.

Similar larvae were recorded by the writer (1946) from fifteen species of host fishes off the west of Ireland. This is the nearest locality to that now under observation from which the larvae have been reported. The three fish hosts in the present instance seem to be new host records.

(4) **Proleptus acutus** Duj., 1845

Synonymy. *Spiroptera dacnodes* Creplin, 1851; *Spiropterina dacnodes* (Creplin 1851) Oerley 1885; *Histiocephalus dacnodes* (Creplin 1851) Molin 1860.

Host. *Raja clavata* L.

Locality. East coast of Iceland, 11° 14′ W., 65° 58′ N.

Depth. 65 fathoms.

Incidence. 19 August 1948, 3 females, 1 male.

Habitat. Intestine.

Proleptus acutus is the type species of the genus *Proleptus* and was first described by Dujardin (1845), who found a single male specimen in the intestine of *Raja clavata* at Rennes. It was later recorded by Diesing (1851) from the same host species, and between 1851 and 1885 was described under three other names as indicated by the synonyms listed above. There are only a few records of the species, and its distribution as far as is known is limited.

In the present instance three females and one male have been recovered from the intestine of *R. clavata* off the coast of Iceland. The length of the single male specimen here recorded is greater than that given by Dujardin for his single specimen, being 16 mm. as compared with 12 mm. The female specimens are 22–30 mm. long. The body in both sexes is broader posteriorly and tapers anteriorly. The male tail terminates in a point and is provided with two rows of large papillae.

ACANTHOCEPHALA

Acanthocephala have been reported on several occasions from marine fishes in various parts of the world. Among those who have recorded them are Linton (1901) at Woods Hole, Lühe (1909) in Europe, Yamaguti (1934–6) in Japan, Baylis & Jones (1933) at Plymouth and Rees (1945) at Aberystwyth.

In the present instance only one species has been found, namely, *Echinorhynchus gadi* Zoega in Müller, 1776. Details of its occurrence are as follows:

Hosts.	*Brosme brosme* (Müll.)	*Platichthyes flesus* (L.)	*Gadus callarias* L.
Locality.	15° 15′ W., 63° 47′ N.	10° 54′ W., 65° 55′ N.	10° 54′ W., 65° 55′ N.
Depth.	160 fathoms	160 fathoms	160 fathoms
Incidence.	21 August 1948, 2 males, 1 female	21 August 1948, 1 male, 1 female	21 August 1948, 2 males, 3 female

Echinorhynchus gadi is a fairly common parasite of fishes and occurs in various parts of the world. Linton (1899) recorded it from nineteen species of host fishes at Woods Hole, among them *Gadus callarias*. Van Cleave (1924) found it to occur in three host fishes also in American waters, among them *G. callarias*, and Yamaguti (1934–6) recorded it from eleven host species in Japan. In Europe Lühe (1909) found it in various Gadidae, *Pleuronectes flesus* and *Acipenser sturio* in the North Sea. Baylis & Jones (1933) recorded it from *Spinachia vulgaris* at Plymouth and Rees (1945) from *Onos mustellus* at Aberystwyth. It is, therefore, widespread in distribution both geographically and among fish hosts.

As far as the writer can ascertain it has not previously been recorded from *Brosme brosme*.

Measurements only of the collected specimens will be given here. The largest female is 25 mm. long by 1·29 mm. broad and the largest male 14 mm. long by 0·95 mm. broad. The proboscis is 0·64–0·96 × 0·26–0·37 mm. and its sheath 1·18–1·56 × 0·23–0·32 mm. There are 18–20 longitudinal rows of hooks on the proboscis, each row containing 10–13 hooks. They are 0·046–0·054 mm. long. The testes are up to 1·9 mm. long and the cement glands 0·83 mm. The fusiform eggs are 0·104 × 0·05 mm. These measurements agree very closely with those given by previous workers.

It is interesting to note that of the parasites in this collection the Monogenea, with one exception, and the Acanthocephalan were found in deep water, while the nematodes were from fishes in more shallow water. Of the three Monogenea, *Linguadactyla molvae* from *Molva byrkelange* and *Diclidophora denticulata* from *Gadus virens* were obtained from depths of 140 and 160 fathoms respectively and *Rajonchocotyloides emarginata* from *Raja clavata* at 160 and 65 fathoms. The only other record of the depth from which Monogenea have been recovered is that of Rees & Llewellyn (1941), when the majority occurred between depths of 120 and 200 fathoms. Three species, all parasites of Elasmobranchs, were obtained from depths of from 0 to 50 fathoms. This is interesting, as it is *Raja clavata* in the present instance which is parasitized at depths of 65 and 160 fathoms.

The four species of nematodes were obtained from shallow water, namely, at depths of from 58 to 68 fathoms. None was found in fishes taken from greater depths. Whether this has any significance is doubtful, as *Contracaecum clavatum* has been found by the writer (1945) in *Cottus bubalis* and *Onos mustellus* in rock pools at Aberystwyth and also (1946) at depths of 50 fathoms in the Irish Sea and 120–150 fathoms on the Irish Atlantic Slope.

Anisakis on *Porrocaecum* larvae have also been recorded by the writer (1946) at depths of up to 200 fathoms.

The three hosts from which *Echinorhynchus gadi* were obtained were all from a depth of 160 fathoms, but depth is probably of little significance here, as the species has been found by the writer (1945) in *Onos mustellus* from rock pools at Aberystwyth.

Whether or not depth has any significance in determining the distribution of parasitic worms of marine fishes cannot be decided until more data have been accumulated.

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A KEY TO THE ETHIOPIAN SPECIES OF THE GENUS *POLYPLAX* (ANOPLURA), WITH DESCRIPTIONS OF TWO NEW SPECIES

By H. E. PATERSON, B.Sc.(Hons.) and P. M. THOMPSON, B.Sc.

Department of Entomology, South African Institute for Medical Research, Johannesburg

(With 12 Figures in the Text)

We believe that the publication of a new key to the species of the genus *Polyplax*, so soon after that contained in Ferris's recent monograph (Ferris, 1951), is justified since it includes two new species and three species, *praomydis* Bedford, *subtaterae* Bedford and *cummingsi* Ferris, which appear to require some re-definition as Ferris placed all three in synonymy.

While sympathizing with Ferris's reaction against the work of many specialists in this group, we believe that the principle can be carried too far and may lead to the ignoring of constant, though slight, differences, which will separate two closely related species or indicate the presence of two subspecies of a single species. It is, of course, very often impossible to decide on the status of a population in the absence of breeding experiments. In all such cases we proposed to consider them good species when the differences are clear-cut, even though slight. The cases of *subtaterae* and *cummingsi* fall, perhaps, into this category.

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I. KEY TO THE ETHIOPIAN SPECIES OF THE GENUS *POLYPLAX*

1 (36) Thoracic sternal plate present **2**

2 (19) Thoracic sternal plate with a narrow, handle-like anterior process **3**

3 (6) Head in both sexes slender, 1·6–1·8 times longer than wide.
Pre-antennal area rounded; antennae set well back from the anterior margin, and not modified in the male. Third paratergal plate with a dorsal seta of about twice the length of the plate. Male genitalia with long parameres reaching to the tip of the pseudopenis **4**

4 (5) Females possess the following combination of characters: tergites 2–3 with setae as follows: 2+6, 6; sternite 2, 5+2; sternites 4–6 each with 7+6 setae. Males possess the following chaetotaxy on sternites 2–6: 6+4, 4+4, 7, 7, 7. (Fig. 12.) Hosts: *Otomys irroratus* Branta from Barotseland, Transvaal, Kenya, Natal; *O. tropicalis* Thomas from Transvaal; *O. angoniensis* Wroughton from Kenya.
otomydis Cummings

5 (4) Females possess the following combination of characters: tergites 2–3 with setae as follows: 2+4, 4; sternite 2, 5+[4+2 weak lateral setae]; sternites 4–6: 5+[4+(2)], [5–6]+[4+(2)], [5–6]+[4+(2)]. Males possess the following chaetotaxy on sternites 2–6: 5+4, 5+4, 6, 6, 6. (For male genitalia see Fig. 11.) Hosts: *Dasymys incomtus* Sund. from Natal and East Africa; *D. nudipes* Peters from Barotseland; *Aethomys chrysophilus* De Winton from Natal.
cummingsi Ferris

6 (3) Head in both sexes more or less broad being from 1–1·4 times longer than broad **7**

7 (8) Setae on all paratergites longer than the plate that bears them, dorsal and ventral setae of about equal length.
Sternal plate oval with prominent 'handle'. Thorax considerably longer than the head and bearing a submarginal row of short setae anterior to the spiracle on the dorsum. Male unknown. Host: '*gros rats*' from Abyssinia.
praecisa (Neumann)

8 (7) Setae on paratergal plates of various lengths **9**

9 (10) Paratergal plate 3, but not 4, with the dorsal seta very long.
Paratergal plates 4–6 with setae which are about as long as the plate bearing them. Head more or less truncate in front of the antennae. Posterior part of the sternal plate oval. Dorsum of thorax with a row of short submarginal setae anterior to the spiracle. Hosts: *Tatera vicina* Peters from East Africa.
taterae Ferris

10 (9) Paratergal plate 3 with or without a long dorsal seta, but paratergal plate 4 with a long seta **11**

11 (18) Paratergal plates 3 and 4 each with a long seta. Other setae on plates 3–6 much shorter 12

12 (13) Ventral side of the abdomen with 2 setae between the paratergal plates and sternite of segments 5–6. Dorsum of thorax with 4 submarginal setae anterior to the long setae. Tergal and sternal plates weakly developed in both sexes. Thoracic sternal plate is triangular with broadly rounded corners. The male has short, thorn-like setae on tergites 4–7 as well as the usual longer setae. Hosts: *Tatera schinzi* Noack from Rhodesia, Ngamiland, Swaziland, Bechuanaland, Barotseland, South West Africa, Orange Free State; *T. böhmi* (Noack) from East Africa; *T. branstii* Smith from Transvaal, Basutoland, Bechuanaland, Barotseland; *T. afra* Gray from Cape Province; *T. liodon* Thomas from Barotseland. **biseriata** Ferris

13 (12) Ventral side of the abdomen with but a single seta between the paratergal plate and the sternite of segments 3–6 14

14 (15) Dorsum of thorax with no submarginal setae anterior to the thoracic spiracle. The third antennal segment of the male not strikingly modified. Sternal plate quite slender, anterior process weak, anterior margin rounded, lateral margins somewhat concave, posterior end blunt—whole posterior part not oval. Tergite 2 bears 2+10 setae in the male and 2+8 in the female. Tergites 3–6 bear about 12–16 setae in the male, and 10–12 in the female. Host: *Gerbillus pyramidium* Geoffroy from Sudan. **gerbilli** Ferris

15 (14) Dorsum of thorax with at least a single, short, submarginal seta anterior to the thoracic spiracle. The third antennal segment strongly modified in the male 16

16 (17) Sternal plate with the posterior part broad, roughly triangular, with rounded corners, small anterior process, anterior margin more or less straight and at right angles to the long axis of the plate, posterior end blunt (Fig. 3). Basal cell of the male antennae elongate, longer than broad (Fig. 2). Tergite 2 with 5+14 setae in the male and 4+12 in the female. Tergites 3–6 with about 15–17 setae in both sexes. Host: *Gerbillus paeba* Smith from South West Africa. **rose-innesi** n.sp.

17 (16) Sternal plate with the posterior part more slender, and the whole oval in shape, the anterior margin sharply rounded and running broadly into the anterior process; the sides of the posterior part convex. Basal cell of the male antenna as long as broad and not at all elongate. Tergite 2 with 6+12 setae in the male and 4+8 in the female. Tergites 3–6 with about 20 setae each in both sexes. Host: *Tatera liodon* Thomas from Uganda. **subtaterae** Bedford

18 (11) Paratergal plate 4 with a long seta, and paratergal plate 3 with two short setae only. Dorsal and ventral setae of each of paratergal plates 5 and 6 of different lengths, all shorter than the length of the plates (Fig. 10). Third antennal segment strongly modified in the male (Fig. 6). Dorsum of thorax with one seta anterior to the spiracle. Host: *Desmodillus auricularis* Smith from South-West Africa. **hopkinsi** n.sp.

19 (2) Thoracic sternal plate without an anterior process 20

20 (21) Paratergal plates of segments 3–6, each with the dorsal posterior angle produced into a slender tapering process which is more than half as long as the plate itself, and with the ventral posterior angle produced into a broader and more blunt process. Sternal plate triangular with rounded corners. Pseudopenis of male bent back in the form of a hook, the parameres exceptionally small. Paratergal plates 7 and 8 are the only ones bearing setae longer than the plates themselves. Hosts: *Rattus tulbergi* Thomas from East Africa; *Rattus coucha* Smith from Rhodesia; '*several rats*' from Transvaal. **waterstoni** Bedford

21 (20) Paratergites with short posterior angles 22

22 (25) Paratergal plates of an unusual form: plates 3–6 large with dorsal margin nearly straight and ventral margin almost semicircular; the posterior margin deeply emarginate or not 23

23 (24) Tergites and sternites of the abdomen occupying the greater part of the abdominal width. Posterior margin of paratergal plates 3–6 deeply emarginate. Host: *Rhabdomys pumilio* Sparrman from Transvaal, Kenya and South West Africa. **avicanthis** Bedford

24 (23) Tergites and sternites of the abdomen are narrow, occupying not more than half of the abdominal width. Posterior margin of the paratergal plates 3–6 only slightly emarginate. Hosts: *Arvicanthis abyssinicus* Rüppel from Uganda; *Otomys tropicalis* Thomas from Uganda; *Oenomys hypoxanthus* Pucheran from Uganda. **abyssinica** Ferris

25 (22) Paratergal plates of usual form with more or less straight dorsal and ventral margins 26

26 (27) Abdominal tergal and sternal plates very short, being shorter than the interstices between the plates. Paratergal plates 3–6 each bearing two equal-sized setae, which are shorter than the plate bearing them. Sternal plate triangular with broadly rounded corners, and rounded anterior margin. Male with strongly modified third antennal segment. Male genitalia with parameres reaching tip of the pseudopenis. Hosts: *Saccostomus campestris* Peters from Natal and South-West Africa. **jonesi** Kellog & Ferris

27 (26) Abdominal tergal and sternal plates well developed, being much longer than the interstices between the plates **28**

28 (31) All paratergal plates bear setae which are at least as long as the plates bearing them **29**

29 (30) Head slender, about twice as long as broad in both sexes.
Antennae set well back on the head; pre-antennal area acutely rounded; sternal plate shield-shaped; male with the antennae weakly modified. Male genitalia with basal plate widely expanded posteriorly; pseudopenis Y-shaped; parameres almost reaching to the tip of the pseudopenis. Hosts: *Acomys cahirinus* Desmarest from Egypt; *A. hystrella* Heller from Uganda; *A. percivali* Dollman from East Africa. **oxyrrhynchus** Cummings

30 (29) Head about as long as broad in both sexes.
A slender species with a relatively small head, much shorter than the thorax. Paratergal plates very slender. Sternal plate shield-shaped with a very short, broad anterior process. Male has antennal third segment strongly modified. Male genitalia has basal plate with almost parallel sides, large parameres which reach the tip of the pseudopenis. Hosts: *Lophuromys aquilus* True from East Africa; *L. sikapusi* Temminck from Uganda; *Lophuromys* sp. from Nyasaland; *Thamnomys ibeanus* Osgood from East Africa. **phthisica** Ferris

31 (28) Not all paratergal plates bear setae which are at least as long as the plates bearing them **32**

32 (35) Both setae of paratergal plate 4 much shorter than the length of this plate **33**

33 (34) Males with the third antennal segment strongly modified; head truncate anterior to the antennae; setae on sternites 2–6 are 5+4, 5+6, 6, 6, 5 and on the second tergite, 2+8; parameres of the genitalia about as long as the pseudopenis. Females with setae on the second sternite (4–5)+(3–4) and on the second tergite 2+6.
Hosts: *Rattus rattus* L. and *R. norvegicus* Berkenhout—records from all over the world. **spinulosa** (Burmeister)

34 (33) Males with the third antennal segment barely modified; head rounded in front of the antennae; setae on sternites 2–6 are (3–4)+4, (3–4)+(4–6), (4–5), 4, (3–4) and on the second tergite 2+6. Parameres of the genitalia much shorter than the pseudopenis. Females with the setae on the second sternite (2–4)+4 and on the second tergite 2+4.
Hosts: *Aethomys namaquensis* Smith from Transvaal, Rhodesia, Swaziland, Barotseland, South-West Africa; *A. chrysophilus* De Winton from South-West Africa. **praomydis** Bedford

35 (32) Dorsal setae of paratergal plate 4 at least as long as the plate itself and very much longer than the ventral seta.
Sternal plate triangular with broadly rounded corners. Male genitalia with parameres much shorter than the pseudopenis; male has the third antennal segment strongly modified. Hosts: *Mus musculus* L.—many records including South Africa. **serrata** (Burmeister)

36 (1) Thoracic sternal plate absent.
Very slender species; abdominal tergites well developed in both sexes and bearing stout, clavate setae. Paratergal plates 3–8 with pairs of long setae. Male antennae strongly modified. Male genitalia with parameres reaching the tip of the pseudopenis. Hosts: *Acomys cahirinus* Desmarest from Egypt; *A. hystrella* Heller from Uganda; *A. percivali* from East Africa. **brachyrrhynchus** Cummings

II. NOTES AND DESCRIPTIONS OF NEW SPECIES

Polyplax rose-inessi n.sp.

Male. Length 1·04 mm. Antennae are set close to the anterior margin of the head, the pre-antennal part of which is truncate. Post-antennal angle quite pronounced, the lateral margins of the hind-head nearly straight and parallel. Occipital region not greatly constricted. Antennae as in Fig. 2. The head is about as long as wide. The thorax is about as long as the head, the lateral margins convex; the sternal plate is shown in Fig. 3. Dorsum of thorax with a short submarginal seta anterior to the spiracle. The claws of the hind-legs are coarse and those of the anterior two pairs much finer. The abdomen is not particularly slender. Paratergal plates (Fig. 5) with the second paratergal plate broad and divided, the ventral piece bearing the longer of the two setae. Paratergal plates 3 and 4 each bear long dorsal setae. Male genitalia shown in Fig. 4; the pseudopenis is hook-like and articulates with the parameres.

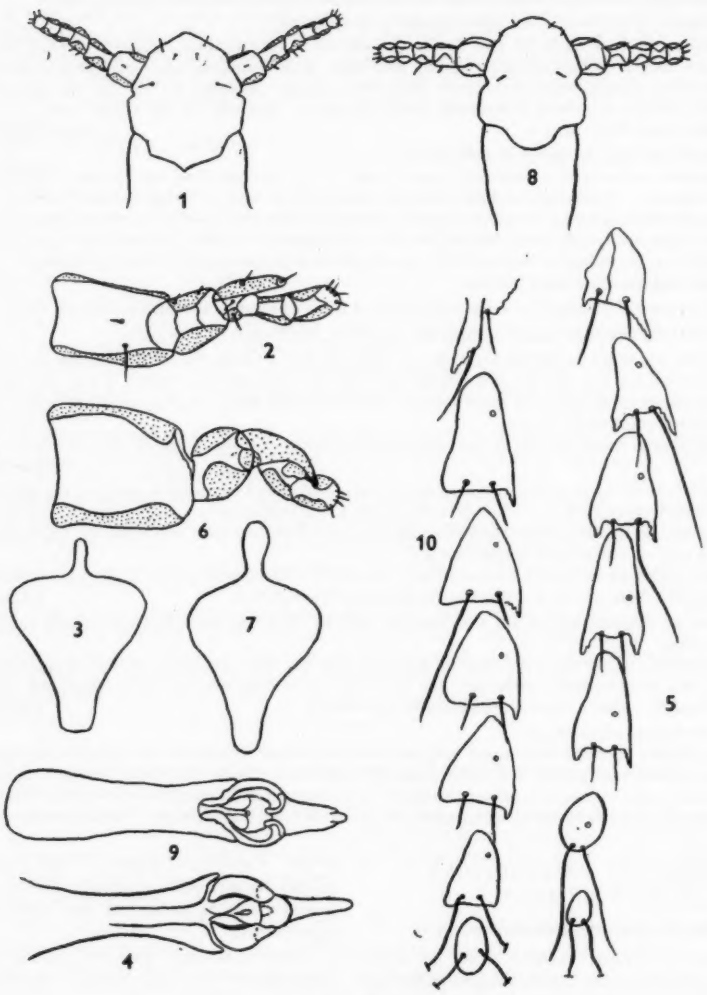
The tergites and sternites are wide but short. The chaetotaxy* of these plates is as follows:

t1	t2	t3	t4	t5	t6	t7	t8
2	5+14	17	21	20	18	16	4

st2	st3	st4	st5	st6	st7	st8
16	18	17	11	12	8	2

Female. Length 1·23–1·45 mm. Head as in Fig. 1; thorax as long as head, and bears a submarginal seta anterior to the spiracle on the dorsum.

* It should be noted that the chaetotaxy of tergites and sternites of *Polyplax* species is never wholly constant. It is often a useful character, however.



Figs. 1–5. *Polyplax rose-innesi* n.sp.

Fig. 1, head of female; fig. 2, antenna of male; fig. 3, sternal plate; fig. 4, male genitalia; fig. 5, left paratergal plates.

Figs. 6–10. *Polyplax hopkinsi* n.sp.

Fig. 6, male antenna; fig. 7, sternal plate; fig. 8, head of female; fig. 9, male genitalia; fig. 10, right paratergal plates.

Chaetotaxy of abdomen as follows:

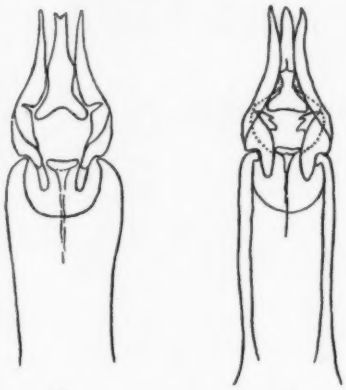
t1	t2	t3	t4	t5	t6	t7	t8
2	4+12	15	16+17	15+16	17+15	13+10	2

st2	st3	st4	st5	st6	st7
18	10+13	12+13	12+13	12+12	9+8

Material. Holotype 1 ♂, paratypes 6 ♀♀. Leg. R. Rose-Innes (Plague Research Laboratory, Johannesburg), Brandberg, South-West Africa, 27 July 1951.

Host. Gerbillus paeba Smith.

Types in the collection of the South African Institute for Medical Research.



11 12

Fig. 11. *Polyplax cummingsi* Ferris. Male genitalia.
Fig. 12. *P. otomydis* Cummings. Male genitalia.

Polyplax hopkinsi n.sp.

Male. Length 1·1 mm. The head is about as long as broad, truncate in front of the antennae which are well set forward. Post-antennal angles prominent, lateral posterior margins of the head straight and nearly parallel, occipital region distinctly constricted. The antennae are shown in fig. 6. Thorax about as long as the head and of usual form with sides convex. Sternal plate as in Fig. 7. The hind claw is blunt, the other two quite slender. A short seta occurs just anterior to the spiracles on the dorsum.

Abdomen not very slender. The tergal and sternal plates are wide but short, their chaetotaxy is as follows:

t1	t2	t3	t4	t5	t6	t7	t8
2	2+10	15	18	14	14	12	4

st2	st3	st4	st5	st6	st7
4+7	9+10	12	13	12	4

Female. Length 1·2–1·4 mm. The head as in Fig. 8. Thorax bears a short submarginal seta anterior to the spiracle on the dorsum. Abdominal tergal and sternal plates wide but short. The chaetotaxy is not clear on the specimens available.

Material. Holotype 1 ♂, paratypes 3 ♀♀, 1 ♂. Leg. R. Rose-Innes. Holotype at 12 miles south of Otjinene, South-West Africa, 25 November 1950. Paratypes 1 ♂, 3 ♀♀ on Luderitz-Aus road, South-West Africa, 15 March 1950.

Hosts. Desmodillus auricularis Smith.

Types in the collection of the South African Institute for Medical Research, Johannesburg.

Note. Mr G. H. E. Hopkins has kindly confirmed the determination of this species and we take pleasure in naming it after him.

Polyplax subtaterae Bedford 1936

After examining a male and two female paratypes of *subtaterae*, and specimens of *taterae* from East Africa off *Tatera vicina*, we consider the species to be distinct. The characters given in the key will separate them.

In the Onderstepoort collection is a male bearing the same data as does the paratype series, but this specimen is somewhat different in that it possesses a submarginal row of setae on the dorsum of the thorax anterior to the spiracle, instead of the single seta possessed by the paratype series. It does, however, have the long dorsal setae on both the 3rd and 4th paratergal plates.

Polyplax taterae Ferris 1923

Polyplax taterae mombassae Werneck = *taterae taterae* Ferris n.syn.

Werneck (1940) was apparently misled by Ferris's erroneous description of the setae on paratergal plate 3 (Ferris, 1923). Ferris has since corrected his earlier error (Ferris, 1951).

I have seen this species off *Tatera vicina* from East Africa.

Polyplax praomydis Bedford 1929

Ferris (1951), after seeing a male of the type series of *praomydis*, synonymized it with *P. spinulosa* Burm., despite the fact that in *praomydis* the male has a slightly modified antenna, differences in abdominal chaetotaxy and differences in the male genitalia and the females show differences in abdominal chaetotaxy. We have seen paratypes of *praomydis* and much other material from the same host. We have, therefore, no hesitation in reinstating *praomydis*.

Polyplax biseriata Ferris 1923

It is interesting to note that *Tatera liodon* from Simakumba, Barotseland were carrying *Polyplax biseriata* Ferris and not *P. subtaterae* Bedford. This

appears to be a case of the geographical distribution of the parasites being independent of the distribution of the host.

Polyplax cummingsi Ferris 1916

We hold the opinion that the characters given in the key are adequate grounds for considering this species to be distinct from *P. otomydis* Cummings. *Dasymys* is a rodent living in the same habitat as does *Otomys*, and thus offering excellent opportunity for a transfer of parasites. This is apparently what has occurred in the case of *Polyplax cummingsi*.

Polyplax otomydis Cummings 1912

The lice from the genera *Parotomys*, *Myotomys* and *Liotomys* appear to show small constant differences from *Polyplax otomydis*. A study of these lice will be made when adequate material is to hand.

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TRANSPORT OF MALLOPHAGA BY FLEAS

By F. G. A. M. SMIT

(With 1 Figure in the Text)

Among a large collection of alcohol material of Danish fleas, belonging to the Universitetets Zoologiske Museum at Copenhagen and kindly sent to me for identification by Dr S. L. Tuxen, was a tube which not only contained the fleas *Chaetopsylla trichosa* Kohaut (6♂, 22 ♀) and *Paraceras melis melis* (Walker) (3 ♂, 30 ♀), but also a large number of the chewing louse *Trichodectes melis* (Fabricius).* These specific badger-parasites were collected on the 27 October 1939 from a specimen of *Meles meles* received by the Department of Vertebrates of the Copenhagen Museum; the badger was caught at Skovshoved (Sealand, Denmark). Whilst separating the fleas from the lice I found it difficult to remove lice from several of the fleas. I then realized that these were cases of phoresy, and was fortunate enough to find in the batch a flea from which I had not yet removed the louse. The flea in question is a female of *Chaetopsylla trichosa* and the louse, a female of *Trichodectes melis*, is firmly attached on the outside of the left mid-tibia by having its mandibles entirely surrounding the tibia (Fig. 1). The two insects have been mounted on one slide (which is incorporated in the British Museum collection of fleas at Tring) and the attachment of the louse to the flea is so firm that the act of preparation did not separate the two insects.

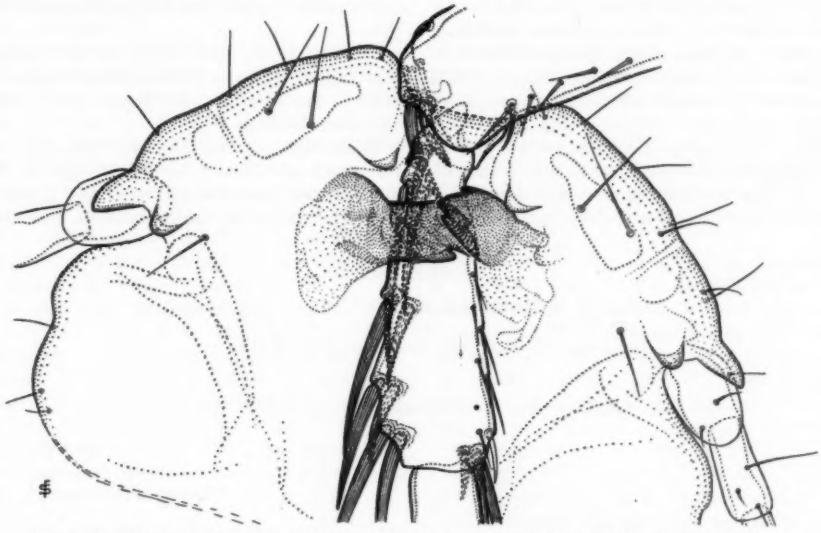

Fig. 1. Head of *Trichodectes melis* attached to the left mid-tibia of *Chaetopsylla trichosa* by means of the mandibles.

Only one other recorded instance of this relationship between Mallophaga and Siphonaptera which is known to me is that recorded by Thompson (1934) who received from the late Prof. Julius Wagner a male of *Paraceras m. melis*, collected by him from a badger in the Crimean Peninsula; 'firmly attached to the hind tibiae of the flea by their mandibles were the head and thorax of a louse and one perfect female. The lice proved to be *Trichodectes melis* Fabricius, ...' (The sex of the 'head and thorax' could have been determined, since the antennae of this louse show a remarkable sexual dimorphism.)

It may well have been that specimens of *Paraceras m. melis* were also among the batch of fleas from

* A list of records of Danish fleas, based upon this material, will be published in the *Entomologiske Meddelelser*.

which I removed lice. In any case, both of the specific badger-fleas (*P. m. melis* and *C. trichosa*) are now known to act, under certain conditions, as a means of transport for the badger-louse. It is of considerable interest that in both instances the lice attached themselves to the tibiae; that particular part of the flea's leg seems to be the most suitable for the louse to cling to, and its thickness may therefore be assumed to be subequal to the thickness of those hairs of the host to which the louse often attaches itself by means of the mandibles.

Most mammal-fleas do not come into contact with Mallophaga, since, for example, insectivores and bats have no Mallophaga and relatively few rodents are hosts to chewing lice. It is remarkable that so far no bird-fleas have been observed to carry Mallophaga, but this may be partly due to the fact that most bird-fleas do not remain for a sufficiently long time on the host after its death, and also, relatively little collecting of fleas from the bodies of birds has been done. Clay & Meinertzhagen (1943) record a number of cases in which Mallophaga were found to be attached to Hippoboscid flies. They carried out several simple experiments which proved that the Mallophaga attached themselves to the flies after the death of the host, thus 'using the flies as a lifeboat'. Since the badger is not a rare animal in the British Isles, it would be of interest to carry out the following experiments, which are similar to those of Clay & Meinertzhagen:

(*a*) In order to establish whether lice are also carried by fleas on living hosts under natural conditions, fleas should be collected from living badgers (after having ascertained that the badgers are also infested with lice) and inspected for attached lice; alternatively, fleas could be collected from a badger immediately after its death, before the animal has had time to cool down.

(*b*) In order to obtain additional proof of lice using fleas 'as a lifeboat', collect all fleas alive from a freshly killed badger and inspect the living fleas for lice; then make sure the host is louse-infested, and put the fleas back on the dead animal, wrapping it up in cloth. After a few hours expose the wrapped-up animal to the fumes of chloroform and after a few minutes collect the fleas and examine them for lice.

(*c*) This is an alternative for (*b*), though much less exact: wrap a freshly killed badger up and chloroform it after a few hours; then collect and examine the fleas.

Note that in the British Isles the only flea occurring specifically on the badger is *Paraceras melis melis*, representatives of the genus *Chaetopsylla* never having been found in these Isles.

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TWO TROMBICULID LARVAE (ACARINA: TROMBIDIIDAE) FROM COSTERMANSVILLE (KIVU PROVINCE, BELGIAN CONGO) OF WHICH ONE IS A NEW SPECIES: *SCHÖNGASTIELLA WANSONI* N.SP.

By J. WOLFS and P. H. VERCAMMEN-GRANDJEAN

(With 7 Figures in the Text)

In August 1952 one of the authors captured a series of rodents of the type *Rattus rattus alexandrinus* at Costermansville. The rats were examined at the Medical Laboratory, Astrida (Ruanda), by kind permission of the Director, Doctor J. B. Jadin. Two species of trombiculids were found in one ear, which was very scabby and contained other microscopic arachnidians.

The first of the two species, *Schöngastia lavoipierri* Jadin & Vercammen (1952) has already been described. Three specimens of this species were found at Astrida on *Oenomys hypoxanthus* and *Arvicanthis abyssinicus*. In this respect two remarks are to be made: (1) the fact that the same species was found in two different places 100 km. apart, and separated by an impenetrable forest 60 km. wide stretching from Kisenyi to Ngozi; and (2) on a host very different from those of Astrida, especially as to its essentially domestic habits.

The second species, represented by an only specimen, is new and will be described below. The fact that these trombiculids were found on a rat of domestic habits has a certain importance regarding typhic incidences. Nevertheless, we must say that up till now, although more than 100 rodents of this species have been examined, only one *Rattus rattus alexandrinus* has been found to be a carrier of trombiculid larvae.

Schöngastia lavoipierri Jadin & Vercammen 1952

Two specimens were found. All morphological and dimensional characters correspond to those of the three specimens found at Astrida. This species is very much like *S. willmanni* Radford (1947).

Host. *Rattus rattus alexandrinus.*

Locality. Costermansville.

Specimens. Two in authors' private collection.

Schöngastiella wansoni n.sp.*

The only specimen of this species was found on *Rattus rattus alexandrinus* on 10 August 1952. This rat, with domestic habits, was captured at Costermansville. Although very much like *Schöngastia dureni* Jadin & Vercammen (1952) the pseudostigmatic organs are quite different. This same character differentiates it from *S. caeca* M. André (1951).

Diagnosis. The segmentations formula of the legs (7.6.6), the shape of the scutum in the form of an elongated pentagon and ornamented by three pairs of marginal barbed setae, the club-shaped pseudostigmatic organs and the chelicera with one dorsal tooth, class it in the genus *Schöngastiella* Hirst, 1946.

Description. Opisthosoma pinkish yellow with posterior edges in the form of a pointed arch. Striae very plain. The measurements of the type and only specimen expressed in microns are shown in Table 1.

Dorsal view. The elongated pentagonal scutum is densely and finely punctuated. The six marginal setae are much alike. The barbs are rare and short (same for the other dorsal setae). The pseudostigmatic organs are club-shaped. The spatulate clubs are more rounded on one side than on the other; their length is four times the breadth. They have long barbs of from 6 to 8 μ. Eyes are absent. The humeral setae are the longest.

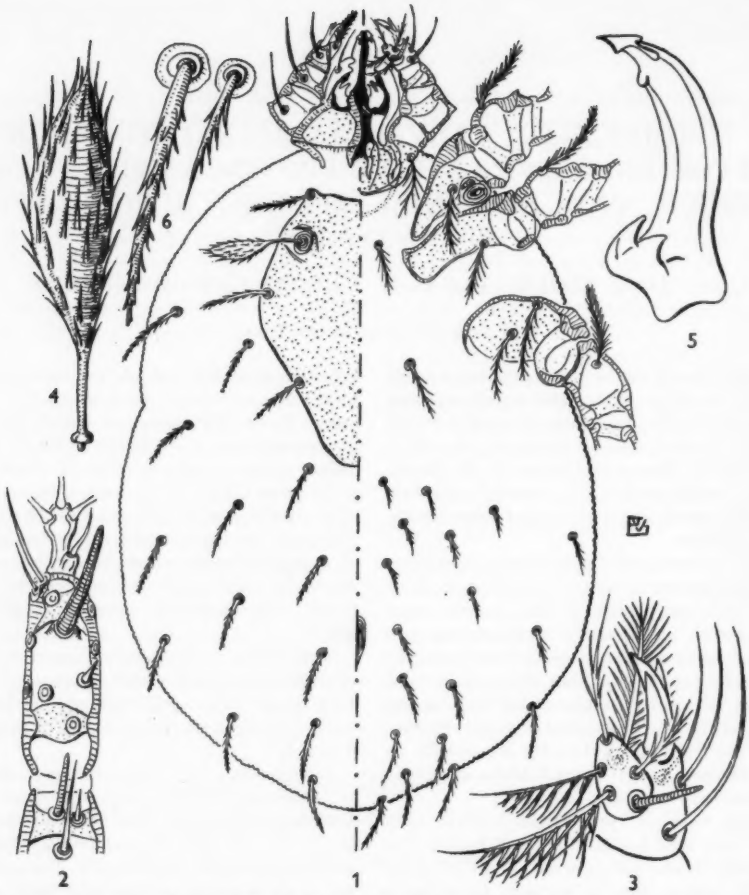
The setae formula is: 2.2.6.6.4.2.2 which, with the two humerals, gives 26 *dorsal setae.*

Ventral view. The gnathosoma has the usual two barbed setae. The barbs of the ventral setae are proportionately long. The intercoxals are the same as the coxals but with longer barbs. The pre-anals are shorter than the post-anals which resemble the dorsals. The uropore is situated at about 80–100 μ from the posterior edge of the opisthosoma, between the setae of the fourth row. The setae formula is: 8.4.6.2.6.4.4.2 which gives 36 ventral setae.

Legs. Segmentation formula: 7.6.6; trochanterial pilose formula: 1.1.1; coxal pilose formula: 1.1.2. On tarsus I the spur is set on a chitinous conical protuberance. The microspur is set laterally behind it. The genu III has a smooth stout seta of middle length.

Buccal apparatus. The galea has a stout smooth seta. The second palpal article presents an acute

* Dedicated to Doctor M. Wanson, Professor of Parasitology at the Institute of Tropical Medicine 'Prince Léopold', Antwerp.



Figs. 1–6. *Schöngastiella wansoni* n.sp. Fig. 1. Left, dorsal view. Right, ventral view. Fig. 2. Tarsus I right. Fig. 3. Ventral view of segments IV and V of palp. Fig. 4. Pseudostigmatic organ. Fig. 5. Chelicera. Fig. 6. Dorsal and ventral setae (pre-anal).

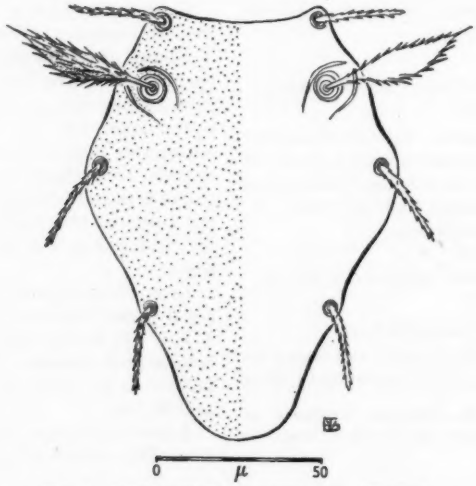


Fig. 7. *Schöngastiella wansoni* n.sp. Scutum.

exterior angle. Dorsally, segments II, III and IV have a smooth seta. Ventrally and laterally segment IV has two other smooth setae. The conical tarsus (segment V) carries: (*a*) terminally—one barbed seta; (*b*) ventrally—one small feathered seta; (*c*) basally—one slender spur; (*d*) laterally—two long barbed setae; (*e*) dorsally—one long feathered seta. The claw is trifid and strong. The chelicera has a rounded dorsal tooth and a tricuspid cap.

Host. Rattus rattus alexandrinus.

Locality. Costermansville.

Specimen. One, type deposited in 'Musée du Congo Belge', Tervuren.

Table 1

LE	130	AW	47	AL	29	H	34	LC	380
1EA	54	PW	85	P	31	V	15/23	1C	285
1EP	93	P′W′	55	P′	27	D	23/25	pa	216
1EP′	62	SB	52	S	41	LPp	48	pm	196
1ES	77	AP	48	LpS	9	LGn	65	pp	238
EA	4	AP′	86	LmS	32	1Gn	80		
ES	24			1mS	9				

Explanation of Letters

LE	Maximum length of scutum.
1EA	Breadth of scutum at the antero-laterals.
1ES	Breadth of scutum at the pseudostigmatic organs.
1EP	Breadth of scutum at the postero-laterals I.
1EP′	Breadth of scutum at the postero-laterals II.
EA	Distance between the anterior scutal edge and the antero-laterals.
ES	Distance between the anterior scutal edge and the pseudostigmata.
AW	Distance between the antero-laterals.
PW	Distance between the postero-laterals I.
P′W′	Distance between the postero-laterals II.
SB	Distance between the pseudostigmatic organs.
AP	Distance between the antero- and postero-laterals I.
AP′	Distance between the antero- and postero-laterals II.
AL	Length of antero-lateral setae.
P	Length of postero-lateral setae I.
P′	Length of postero-lateral setae II.
S	Total length of pseudostigmatic organs.
LpS	Length of peduncle of pseudostigmatic organs.
LmS	Length of club.
1mS	Breadth of club.
H	Length of humeral setae.
V	Greatest length of ventral setae.
D	Greatest length of dorsal setae.
LPp	Length of palp.
LGn	Length of gnathosoma.
1Gn	Breadth of gnathosoma.
LC	Length of idiosoma.
1C	Breadth of idiosoma.
pa	Length of anterior legs.
pm	Length of middle legs.
pp	Length of posterior legs.

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FOUR NEW SPECIES OF 'HARVEST MITE' OR 'CHIGGER' AND A NEW FUR-MITE (ACARINA: TROMBICULIDAE AND LISTROPHORIDAE)

By CHARLES D. RADFORD

Membre Correspondant, Muséum National d'Histoire Naturelle, Paris

(With 15 Figures in the Text)

INTRODUCTION

From the Virus Research Institute, Entebbe, Uganda, the author received a number of tubes containing mites collected from small mammals in the course of yellow fever investigations. One of the tubes with mites taken from a hyrax (*Procavia abyssinica meneliki* Neumann) contained two new species of larval Trombiculidae which are described in this paper under the names *Schöngastia haddowi* sp.n. and *Eutrombicula lumsdeni* n.sp. in honour of the donors, Drs A. J. Haddow and Russell M. Lumsden.

My friend and wartime colleague, Major S. L. Kalra, I.A.M.C., of the Indian Army Typhus Unit has, amongst a number of other specimens, sent me the mite described in this paper under the name *Euschöngastia schlugeri* n.sp. in recognition of the work done by Dr E. G. Schluger on the larval Trombiculidae collected from hosts in Siberia, some species of which have recently been found to be parasitic on hosts in Korea.

The fourth species of larval trombiculid mite described as *Eutrombicula watsoni* n.sp. was taken from a fruit bat (*Rousettus aegyptiacus* (Geoffroy, 1810)) at Karavas, Cyprus, and is named in honour of the donor, J. S. Watson.

Dr Harvey B. Morlan, Sanitarian, of the U.S. Public Health Service, Santa Fé, New Mexico, U.S.A., has sent a batch of mites collected from the fur of a rat (*Dipodomys spectabilis*). This mite is described under the name *Listrophorus dipodomius* n.sp.

I wish to express my thanks to the above-named colleagues for their kindness in sending me the material for this paper.

Family TROMBICULIDAE Ewing, 1944

Subfamily Trombiculinae Ewing, 1929

Genus **Schöngastia** Oudemans, 1910

Schöngastia haddowi n.sp.

The dorsal scutum (Fig. 1) of the larva is pentagonal in shape. The anterior edge of the scutum is straight; the lateral edges concave, and the posterior edge is broadly rounded medially. The antero-lateral spines are long, stout and feathered; the antero-median spine is almost half the length of the antero-lateral spines but not so stout and is placed posterior of a line through the antero-lateral spines; the postero-lateral spines are long and feathered, but not as long as the antero-laterals, nor as stout. The postero-lateral spines are wider apart than the antero-laterals. The sensory spine bases are close together and slightly anterior of a line between the postero-lateral spines. Anterior and posterior of these bases are chitinized ridges. The sensory spines are globose, capitate borne on slender peduncles. The surface of the dorsal scutum is pitted. Flanking the dorsal scutum are the eye plates; the anterior eye is level with the postero-lateral spine; the posterior eye being smaller than the anterior eye.

The palpal claw is tridentate; femoral seta nude; genual seta nude; dorsal tibial seta nude. Galeal seta nude (Fig. 2). Cheliceral blade with four dorsal teeth (Fig. 3). All legs with seven segments; tarsi with two claws and a long slender empodium; mastitarsala present on tarsus iii. Spur on tarsus i (Fig. 4) about one-quarter the length of the tarsus. One spine on each coxa.

Dorsal setae about 32, arranged in transverse rows of 2, 8, 6, 2, 4, 6, 4. Ventral setae about 38, in rows of 2, 2, 8, 2, 4, 6, 4, 6, 4.

Standard data. AW 69 μ; PW 95 μ; SB 20 μ; ASB 28 μ; PSB 37 μ; A–P 32 μ; AM 40 μ; AL 77 μ; PL 57 μ; Sens 32 μ; DS 40 μ; SD 70 μ.

Host. A hyrax (*Procavia abyssinica meneliki* Neumann).

Locality. Kaabong, Uganda. 1952.

Genus **Euschöngastia** Ewing, 1938

Euschöngastia schlugeri n.sp.

The dorsal scutum (Fig. 5) of the larva is rectangular; the anterior edge of the scutum is almost straight; the lateral edge is concave; the posterior edge is broadly rounded. The antero-

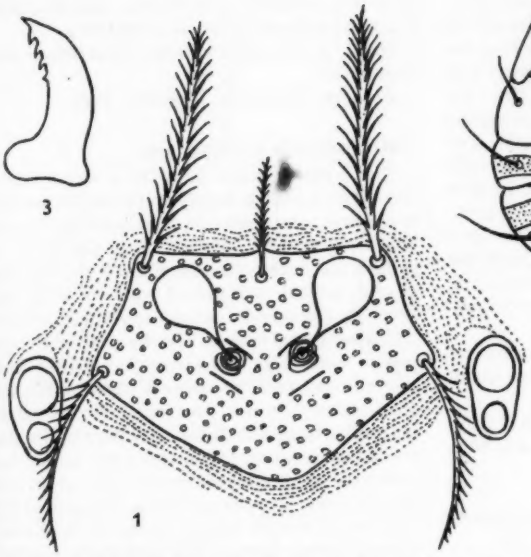


Fig. 1. *Schöngastia haddowi* n.sp. Dorsal scutum of larva.
Fig. 2. *S. haddowi* n.sp. Left palp. Dorsal aspect.

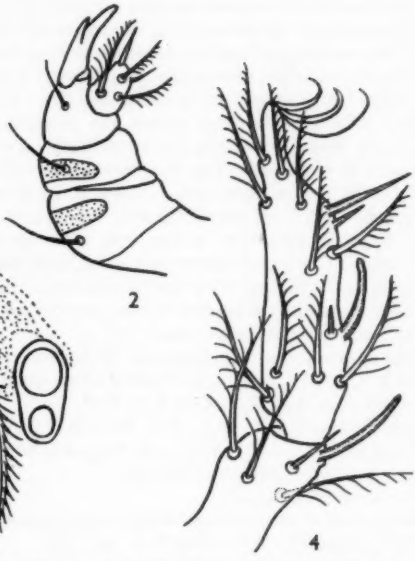


Fig. 3. *S. haddowi* n.sp. Cheliceral blade.
Fig. 4. *S. haddowi* n.sp. Tarsus i of larva.

Fig. 5. *Euschöngastia schlugeri* n.sp. Dorsal scutum of larva.

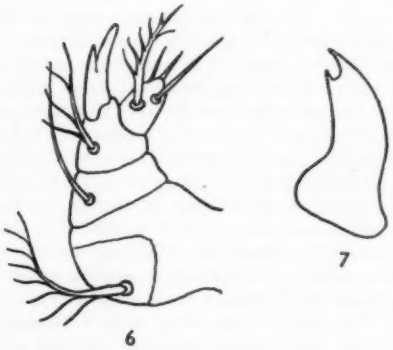


Fig. 6. *E. schlugeri* n.sp. Left palp. Dorsal aspect.
Fig. 7. *E. schlugeri* n.sp. Cheliceral blade.

lateral spines are long, feathered and slightly anterior of a line through the antero-median spine; the postero-lateral spines are long, feathered and as long as the antero-median spine. Surrounding the sensory spine bases are oval ridges, the anterior half of the ridge being more heavily chitinized than the posterior half. The sensory spines are elongate, clavate and furnished with a few short barbs. The palpal claw is tridentate (Fig. 6) femoral seta with lateral barbs; genual seta nude; dorsal tibial seta with lateral barbs; lateral tibial seta with barbs; ventral tibial seta barbed. Galeal seta with three lateral barbs. Cheliceral blade with a single dorsal tooth (Fig. 7). All legs with seven segments; all tarsi with a pair of claws and a long slender empodium. One spine on each coxa.

Dorsal setae about 60, arranged in transverse rows of 2, 8, 12, 12, 6, 8, 8, 4. Ventral setae about 56, in rows of 2, 2, 8, 8, 4, 4, 8, 8, 6, 4, 2.

Standard data. AW 80 μ; PW 87 μ; SB 24 μ; ASB 20 μ; PSB Nil; A–P 30 μ; AM 65 μ; AL 50 μ; PL 65 μ; Sens 44 μ; DS 65 μ; SD 58 μ.

Host. A rat (*Rattus* sp.).

Locality. Chedleth Forest, India. 31 August 1947. S. L. Kalra.

Genus **Eutrombicula** Ewing, 1938

Eutrombicula lumsdeni n.sp.

The dorsal scutum (Fig. 8) of the larva is roughly square, the anterior and posterior edges rounded. The antero-lateral spines are placed some distance from the anterior edge of the scutum; antero-median spine close to the anterior edge of the scutum; the postero-lateral spines as wide apart as the antero-lateral spines. The antero-median spine and the postero-lateral spines are of equal length; the antero-lateral spines are shorter; all five scutal spines are feathered. The bases of the sensory spines are wider apart than the distance between the base and the lateral edge of the scutum, and closer to the anterior than the posterior edge of the scutum. The sensory spines are long, slender, flagelliform with lateral barbs in the distal half. Flanking the scutum are the eye plates, level with and close to the postero-lateral spines; the posterior eye is smaller than the anterior. The palpal claw (Fig. 9) is tridentate; femoral seta with lateral branches; genual seta branched; dorsal tibial seta branched. Galeal seta with lateral branches. Cheliceral blade with a single dorsal tooth and a tricuspid cap (Fig. 10). All legs with seven segments; all tarsi with two claws and a long, slender empodium (Fig. 11). Tarsal spur short. Mastitarsala present on tarsus iii. One spine on each coxa.

Dorsal setae about 40, arranged in transverse rows of 2, 8, 6, 2, 8, 6, 4, 4. Ventral setae about 34, in rows of 2, 2, 6, 8, 8, 4, 2, 2.

Standard data. AW 78 μ; PW 78 μ; SB 32 μ; ASB 28 μ; PSB 20 μ; A–P 30 μ; AM 40 μ AL 32 μ; PL 40 μ; Sens 62 μ; DS 40 μ; SD 70 μ.

Host. A hyrax (*Procavia abyssinicus menelikii* Neumann).

Locality. Kaabong, Uganda. 1952.

Eutrombicula watsoni n.sp.

The dorsal scutum (Fig. 12) is wider than long; the anterior edge is deeply concave; the lateral edge is concave; the posterior edge convex. The antero-lateral spines are shorter than the other scutal spines; the antero-median spine is shorter than the postero-laterals and placed posterior to the antero-laterals; the postero-lateral spines are longer than the other scutal spines. The sensory spine bases are placed well apart and about equidistant from one another and from the lateral edges of the scutum. The sensory spines are long, slender and with lateral barbs on the distal half. Eye plates flanking the dorsal scutum; the anterior eye larger than the posterior and placed above the level of the postero-lateral spine. The palpal claw is tridentate (Fig. 13): femoral seta with lateral barbs; genual seta with lateral barbs; dorsal tibial seta nude. Galeal seta. Cheliceral blade with a tricuspid cap (Fig. 14). All legs with seven segments; tarsi with two claws and a long, slender empodium. Mastitarsala present on tarsus iii. One spine on each coxa.

Dorsal setae about 42, arranged in transverse rows of 4, 8, 10, 8, 6, 4, 2. Ventral setae about 38, in rows of 2, 2, 10, 6, 6, 6, 4, 2.

Standard data. AW 85 μ; PW 112 μ; SB 43 μ; ASB 30 μ; PSB 25 μ; A–P 30 μ; AM 60 μ; AL 52 μ; PL 78 μ; Sens 80 μ; DS 52 μ; SD 56 μ.

Host. A fruit-bat (*Rousettus aegyptiacus* (Geoffroy 1810)).

Locality. Karavas, Cyprus. 12 March 1948. J. S. Watson.

Genus **Listrophorus** Pagenstecher, 1861

Listrophorus dipodomius n.sp.

Differing from other species of the genus in the shape of the caudal lobes and terminal spines of the male. The female is not readily distinguishable from that of other species of the genus.

The male is elongate; legs i placed on the anterior third of the body; legs ii placed midway between legs i and middle of the body; legs iii and iv are placed on the fore part of the posterior half of the body; distal end of tarsus iii and iv truncate, not tapering. Chitinized platelets surround the coxae of all legs. The anterior portion of the body has a cowl-like flap round the capitulum. All legs are without claws or modification of the tarsus for grasping the fur of the host, but provided with a

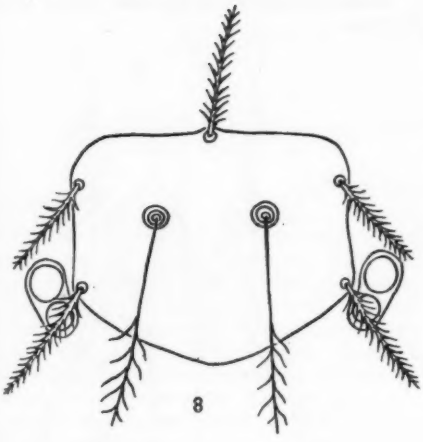
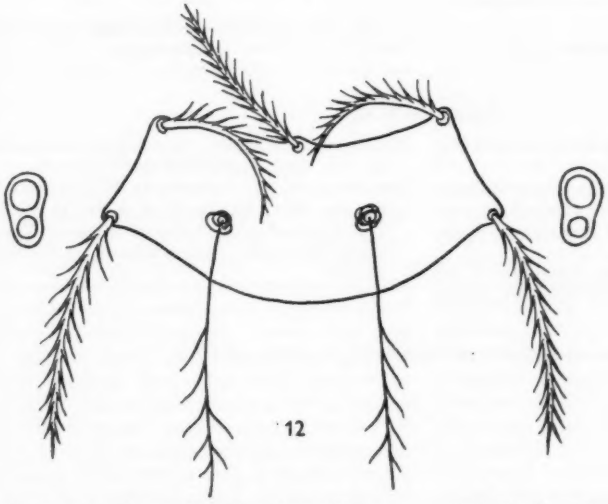


Fig. 8. *Eutrombicula lumsdeni* n.sp. Dorsal scutum of larva.
Fig. 9. *E. lumsdeni* n.sp. Left palp. Dorsal aspect.
Fig. 10. *E. lumsdeni* n.sp. Cheliceral blade.
Fig. 11. *E. lumsdeni* n.sp. Tarsus i of larva.



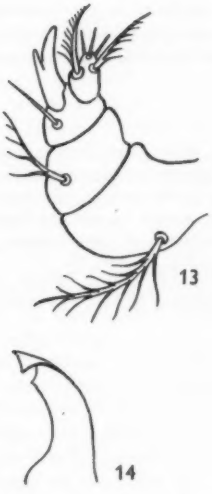


Fig. 12. *E. watsoni* n.sp. Dorsal scutum of larva.
Fig. 13. *E. watsoni* n.sp. Left palp. Dorsal aspect.
Fig. 14. *E. watsoni* n.sp. Cheliceral blade.

tarsal sucker. The genital pore lies between coxae iii and iv and is surrounded by a sclerotized plate. On the dorsal surface there is a chitinized shield anteriorly, extending from the cowl-like flap to the level of coxa ii. The middle of the dorsum is unprotected and the dorsal shield extends from a line between coxae iii and iv to the posterior end of the caudal lobes.

The caudal lobes of the male (Fig. 15) are broadly triangular, surrounded on the outer and inner flank by a hyaline membrane. At the apex of each lobe there is a long, stout spine; flanking this externally is a shorter and thicker spine which has a hyaline blade on the inner edge; anterior and exterior to this there is an extremely long, stout spine. Anterior to these three terminal spines is a pair of shorter spines, the anterior spine being placed upon a chitinized peduncle. Some distance anterior to this pair of spines and on the lateral edge of the body is another spine. Close to the median line and anterior to this last caudal spine are the genital pair of suckers with the genital pore and penis anterior to them. The specimens are mounted in the lateral position, and it is not possible to make a complete figure of the dorso-ventral aspect.

Female not unlike the females of other species in this genus. The dorsum is provided with but one chitinized shield, which covers the anterior portion of the body from the cowl-like flap posteriorly to the level of coxae ii.

Host. A rat (*Dipodomys spectabilis* spp.).

Locality. Santa Fe, New Mexico, U.S.A. 11 October 1951. Harvey B. Morlan.

Measurements. ♂, 0·71 × 0·41 mm.; ♀, 0·45 × 0·11 mm.

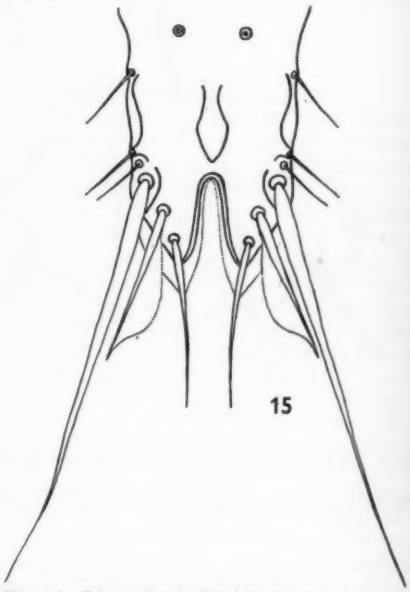


Fig. 15. *Listrophorus dipodomius* n.sp. Caudal lobes of male.

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DIPETALONEMA SPRENTI N.SP. FROM *CASTOR CANADENSIS* KUHL

By ROY C. ANDERSON

Department of Parasitology, Ontario Research Foundation, Canada

(With 12 Figures in the Text)

On 26 August 1948 Dr J. F. A. Sprent collected a female specimen of *Dipetalonema* sp. from the peritoneal cavity of a beaver (*Castor canadensis*) from Ontario (exact locality unknown). Female specimens were subsequently collected from the peritoneal cavity of two other beavers, one from Algonquin Park, Ontario (six female specimens) and another from Wylie Township (one female specimen). On 21 January 1953 Mr C. D. Fowle, of the Ontario Department of Lands and Forests, kindly presented to the writer four more female and two male specimens removed from the body cavity of a beaver from Algonquin Park, Ontario, by Miss B. M. Caldwell and Mr T. Cusson, also of the Department of Lands and Forests. The recent discovery of male specimens makes a systematic study of this nematode possible. This filariid, although similar to *Dipetalonema arbuta* Highby, 1943, appears to be a new species and it is described herein and named in honour of Dr J. F. A. Sprent who collected the first specimens.

Dipetalonema sprenti n.sp.

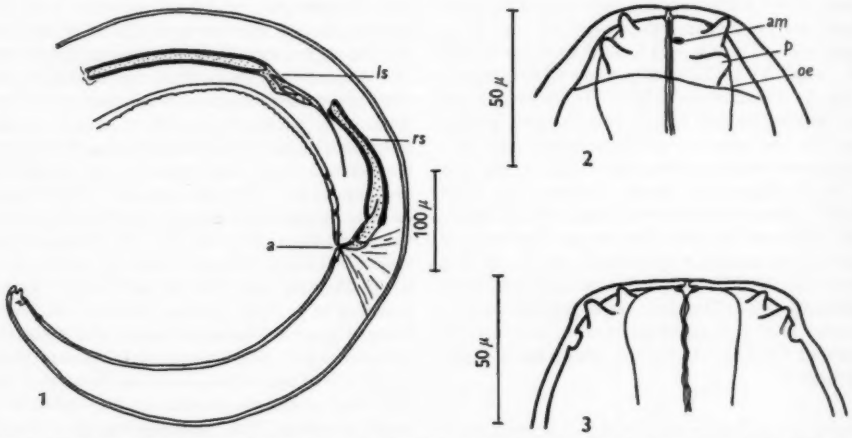
(Figs. 1–4, 6–7, 9–12)

Description. Dipetalonematidae Wehr, 1935; Dipetalonematinae Wehr, 1935; *Dipetalonema* Diesing, 1861. The body is long and attenuated, tapering markedly at both extremities. The anterior end is provided with a rectangular, cuticular shield (specialized type of Chabaud, 1952). The oral papillae are prominent, consisting of four pairs of submedian papillae. The outer four papillae are larger and located at the corners of the cuticular shield. Internolateral papillae (see Chitwood & Chitwood, 1950, p. 63) are apparently absent. The oral opening, which opens into a small, narrow buccal capsule, is minute, round, and bounded by a delicate circumoral membrane. The oesophagus is divided into a short muscular portion and a longer glandular portion. The cuticle appears smooth except under high magnification when a delicate transverse striation can be seen.

Male (two specimens). The males are 40 and 43 mm. long. The caudal end is loosely coiled three times. The lateral width of the head is 0·080 and 0·084 mm. The body is 0·12 and 0·13 mm. thick at the nerve ring, and 0·068 and 0·072 mm. wide at the anus. The maximum width of the worm, 0·23 and 0·27 mm., is attained near the middle of the body. The oesophagus, which is 2·2 mm. long in both specimens, enlarges to a width of 0·036 mm. immediately behind the oral opening. The nerve ring is 0·34 and 0·36 mm. from the anterior end. The deirids are ventro-lateral in position; one (0·62 and 0·63 mm. from the anterior end) occurs more anteriorly than the other (0·65 and 0·67 mm. from the anterior end). The spicules are unequal in size and shape. The right spicule, which is 0·17 mm. long in both specimens, consists proximally of a short thick calomus. The distal part (lamina?) of this spicule is broad, flat and complex, ending in a blunt hook. The left spicule (0·27 mm. in both specimens) consists of a long tubular calomus and a broad, flanged lamina which continues as a delicate whip-like structure. Neither spicule has a capitulum. The anus is 0·40 and 0·46 mm. from the tip of the tail. The anal opening is slit-like and bounded by a clear band of cuticle. The anal papillae are of two types; four pairs of pedunculate, pre-anal papillae occurring laterally on the body and a single row of four sessile, ventral papillae behind the anus. A small unidentified oval or crescent-shaped structure occurs ventrally, about 17 μ in front of the anal opening. This structure may be a double papilla. The ventral surface of the cuticle in the caudal region has transverse, longitudinally striated ridges. These ridges, which extend up to the end of the third coil, gradually increase in size and become farther apart from the anus to the third coil. Behind the anus the ridge are small, ill-defined and spaced only a few microns apart. The muscular body wall can be seen to extend into the bases of the more prominent ridges. The coiling of the tail results in the ridges always appearing on the lesser curvature of the coils when the caudal region is mounted under a coverslip. The tail terminates in a blunt point and is provided with two ventro-lateral wing-like structures. These structures are cuticular with tongues of tissue from the body wall extending into them. A pair of large, cup-shaped papillae occur ventrally about 35 μ from the tail end. A pair of blunt, ventro-

lateral papillae occur anterior to the wing-like appendages; the left one is slightly anterior to the other. The phasmids are distinct, occurring ventro-laterally behind the most posterior papillae. The post-deirids, which are prominent, appear to be ventro-lateral in position. They occur about 3·5 mm. from the tail-end in the region of the second coil.

Female (twelve specimens). The female is 84–109 (99) mm. long. The caudal end is coiled twice. The lateral width of the head is 0·088–0·11 (0·10) mm. The body is 0·14–0·17 (0·15) mm. wide at the nerve ring, 0·21–0·30 (0·26) mm. wide at the posterior end of the oesophagus and 0·10–0·12 (0·11) mm. wide at the anus. The maximum width of the body 0·49–0·70 (0·58) mm., occurs near the middle of the length, although in one specimen it is about 4 mm. long. In general it appears to be highly variable in length. The anterior part of the vagina is thick-walled and muscular; the lumen is narrow. Posteriorly the wall of the vagina gradually loses its muscular appearance until, well behind the junction of the oesophagus and intestine, it is thin and transparent, although still retaining its cuticular lining. Posteriorly the vagina continues into a thin-walled, transparent, bulb-like sac, from the posterior end of which arise two voluminous uteri. The uteri, which are opisthodelphic, highly coiled and convoluted are packed with embryos in various stages of development. Well-developed embryos are found posteriorly in the uteri, coiled within a clear



Figs. 1–3. *Dipetalonema sprenti* n.sp. Fig. 1. Caudal end male, left lateral view. Fig. 2. Anterior end female, dorsal view. Fig. 3. Anterior end female, lateral view.

worm. The oesophagus is variable in length measuring, 1·7–2·7 (2·4) mm. long. It is swollen immediately behind the oral opening where it is 0·042–0·060 (0·047) mm. wide. The maximum width of the oesophagus is 0·060–0·080 (0·070) mm. The division of the oesophagus into two portions was observed in only four of the twelve specimens; in these the anterior muscular part is 0·63–0·72 (0·68) mm. long and the posterior glandular part is 1·6–2·0 (1·8) mm. long. The nerve ring is 0·27–0·34 (0·32) mm. and the minute excretory pore 0·38–0·49 (0·45) mm. from the anterior end. The deirids are ventro-lateral in position; the right, 0·44–0·63 (0·54) mm. from the anterior end, always occurring slightly anterior to the left which is 0·51–0·62 (0·59) mm. from the anterior end. The slit-like vulva is 0·73–1·1 (0·86) mm. from the anterior end. The vulva leads into a vagina, which is often so highly coiled about the oesophagus and intestine that it is difficult to determine its exact delicate, oval (rarely almost circular) membrane. The greater diameter of these eggs is 48–55 (52) μ and the lesser diameter 40–45 (43) μ. The uteri more anteriorly contain embryos in the process of stretching the enveloping membrane. The most fully developed embryos occur in the vagina, the sac behind the vagina, and in the anterior parts of the uteri. These embryos are sheathed and measure 400–450 (425) μ long and 8 μ wide. The anterior end of the embryo is round and blunt; the posterior end tapers to a fine point. The intestine of the female worm is narrow except for an enlargement of 0·073–0·16 (0·11) mm. immediately behind the oesophagus. The tail, which is 0·39–0·68 (0·48) mm. long, ends in a very small conical projection and is provided with a pair of reduced wing-like appendages. The phasmids are prominent and occur ventro-laterally in front of the lateral appendages on the tail end.

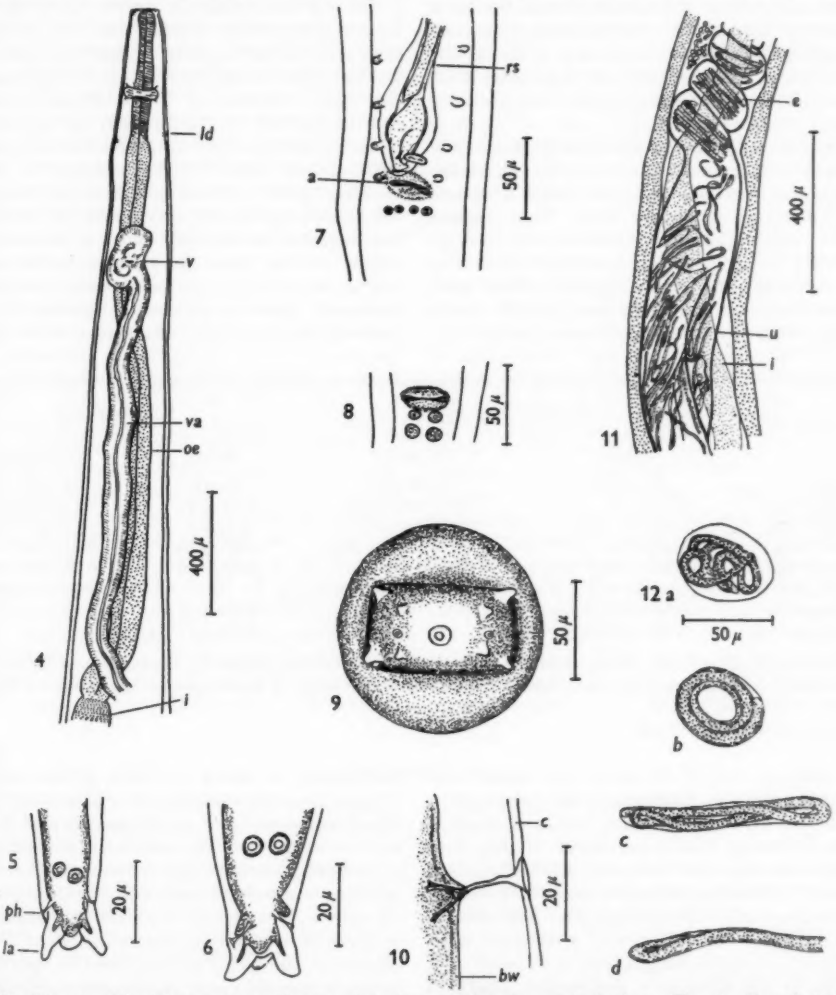
HOST. *Castor canadensis* Kuhl.

LOCATION. Peritoneal cavity.

LOCALITY. Algonquin Park and Wylie Township, Ontario, Canada.

TYPE SPECIMENS. Zoological Division, United States Bureau of Animal Industry.

review of the nematodes of the Rodentia, Lagomorpha and Hyracoidea, suggests that the worms observed by Morgan belong to the genus *Setaria*. Harkema (1936) and Erickson (1944), in their reviews of the parasites of beaver, allude to the



Figs. 4–12. Fig. 4. *Dipetalonema sprenti* n.sp. Anterior end female, ventral view. Fig. 5. *D. arbuta* Highby, 1943. Caudal end male, ventral view. Fig. 6. *D. sprenti* n.sp. Caudal end male, ventral view. Fig. 7. *D. sprenti* n.sp. Anal region of male, ventral view. Fig. 8. *D. arbuta* Highby, 1943. Anal region of male, ventral view. Fig. 9. *D. sprenti* n.sp. Anterior end female, *en face* view. Fig. 10. *D. sprenti* n.sp. Post-deirid. Fig. 11. *D. sprenti* n.sp. Region of bifurcation of uteri. Fig. 12. *a–d. D. sprenti* n.sp. Stages in the stretching of the egg envelope. Embryos dissected from vagina and uteri.

Morgan (1868) recorded (p. 73) that 'large numbers of a long, slender, white worm, 3″ to 5″ in length, were found in the peritoneal cavity (*Filaria* species not known)', of a beaver. Hall (1916), in his early statements of Morgan and Hall regarding a filarioid nematode of the beaver. It is possible that the worms observed by Morgan were *Dipetalonema sprenti* n.sp.

Chabaud (1952), in a recent review of the genus *Dipetalonema* Diesing, 1861, lists twelve species from the Marsupialia (three considered by Chabaud insufficiently described, one of which is unnamed), two species from the Edentata, four from the Ungulata, three from the Carnivora and ten from the Primates (four species insufficiently described, one of which is unnamed). From a study of the descriptions in the literature it is evident that *D. sprenti* is readily distinguished from all these species.

Five species are described from the Rodentia and the Insectivora, namely: Insectivora—*D. weissi* (Seurat, 1914) from *Elephantulus deserti* (Algeria) and *D. sunci* Sandground, 1923, from *Suncus caeruleus* (Indo-China); Rodentia—*D. finlayi* (Mazza & Fiora, 1932) from *Lagidium tucumanus* (South America), *D. arbuta* Highby, 1943, from *Erethizon dorsatum* (North America) and *D. blanci* Chabaud, 1952, from *Meriones libycus* (Iran).

writer has examined the type material (U.S.N.M. Helm. Coll. Nos. 45037 and 45038) of *D. arbuta* as well as other specimens he has collected from Ontario porcupines which agree in all particulars with the type material.

Table 1 shows that *D. sprenti* is on the whole a longer and thicker species than *D. arbuta*. This difference is particularly apparent when one has the two species side by side. It is significant that the type material on which Highby based his description and the specimens from the beaver are all fully mature. Moreover, the writer has measured two male and three female specimens of *D. arbuta* removed from a porcupine which had been maintained in captivity for over 9 months. This animal was captured as an adult and was harbouring *Mf. arbuta* at that time. The dimensions of the adult worms, removed 9 months after the porcupine was captured, agree in all particulars with the data given by Highby (1943). This suggests that Highby's

Table 1. *Comparison (in mm.) of* Dipetalonema arbuta *Highby*, 1943, *with* D. sprenti *n.sp.*

	D. arbuta Highby*		*D. sprenti* n.sp.	
	Male	Female	Male	Female
Length	22–37 (31)	58–82 (68)	40–43	84–109 (99)
Width (max.)	0·17–0·21	0·25–0·33 (0·27)	0·23–0·27	0·49–0·70 (0·58)
Length oes.	2·2–2·7 (2·4)	2·1–2·8 (2·4)	2·2–2·2	1·7–2·7 (2·4)
Nerve ring†	0·20–0·24	0·19–0·26 (0·23)	0·36–0·34	0·27–0·34 (0·32)
Length tail	0·23–0·33 (0·27)	0·20–0·25	0·40–0·46	0·39–0·68 (0·48)
Vulva†	—	0·45–0·85 (0·70)	—	0·73–1·1 (0·86)
Left spicule	0·21–0·22 (0·22)	—	0·27–0·27	—
Right spicule	0·11–0·12 (0·12)	—	0·17–0·17	—

* Data from Highby, 1943, obtained from measuring six male and six female specimens except for body length which is based on ten males and twenty females. Most figures reduced to the second decimal place by the present author.

† Distance from anterior end.

The anterior end of *D. weissi*, *D. finlayi* and *D. blanci* is primitive, lacking a broad, rectangular, cuticular shield, whereas that of *D. sprenti* is specialized, having this broad shield (cf. Fig. 9 of this paper and fig. 1 of Chabaud, 1952). This is a difference of fundamental importance which readily separates *D. sprenti* from these species. Sandground (1932) could find 'no evidence of a flattened cuticular shield...' at the anterior end of *D. sunci*. On the basis of Sandground's description Chabaud thinks that the cephalic end of this species is probably primitive. Even if we regard the cephalic characteristics of this species as unsettled, there are other differences which readily distinguish it from *D. sprenti*, e.g. lengths and morphology of the spicules; the lack of the four median post-anal papillae found in *D. sprenti*; the greater length of the male tail of *D. sunci* (100 μ), etc.

D. sprenti is similar to *D. arbuta*, but there are important specific differences between them. The description is based on fully grown specimens. Under these circumstances the differences found in the dimensions between *D. sprenti* and *D. arbuta* may be significant. The position of the nerve ring is consistently further (approximately 100 μ) from the anterior end in both male and female specimens of *D. sprenti*. The tail of *D. sprenti*, especially of the females, is markedly longer than that of *D. arbuta*. Moreover, the spicules, considered by many authors to be of specific value in the genus *Dipetalonema*, are considerably longer in the two male specimens from the beaver (right spicule approximately 42% longer; the left approximately 23% longer).

There appears to be a morphological difference between the two species as well. There is a *single row* of four median papillae behind the anus in the male specimens of *D. sprenti* (Fig. 7). In the specimens of *D. arbuta* examined by the writer these papillae occur as two pairs, *one pair behind the other* (Fig. 8); this is the arrangement illustrated by Highby.

Caudal papillae in *Dipetalonema* can be expected to vary somewhat both in position and number, and the constancy of the arrangements outlined above for the two species will have to be determined by examining more specimens as they become available. It is worth noting here that some of the specimens of *D. arbuta* lack the pair of ventro-lateral papillae immediately anterior to the phasmids (Fig. 5). These papillae occur on the male type specimen of *D. arbuta*, although Highby does not mention them.

Female specimens of *D. sprenti* and *D. arbuta* were placed in a mixture of 70% alcohol and 5% glycerine. This mixture was allowed to evaporate until the worms remained in pure glycerine. Fully developed embryos were dissected from the vagina and upper parts of the uteri and eggs from the more posterior regions. The eggs and embryos of the two species were compared.

The eggs and fully developed embryos of *D. sprenti* are much larger than those of *D. arbuta*. The greater and lesser diameter of the eggs of the two species are respectively as follows: *D. arbuta*, 32–40 (37) μ and 21–29 (24) μ; *D. sprenti*, 48–55 (52) μ and 40–45 (43) μ. There is, moreover, a difference of about 130 μ in the length and 2 μ difference in width between the embryos of the two species. Highby gives the size of twenty microfilariae (presumably from the blood of a porcupine) in Aqueous Azur II as 0·280–0·297 (0·288) mm. × 0·006 mm. What appear to be fully developed embryos in the vagina and anterior parts of the uteri of specimens of *D. arbuta* from Ontario measure 0·251–0·282 mm. × 0·006 mm. after gradually evaporating them down to pure glycerine. Fully developed embryos from the vagina and uteri of specimens of *D. sprenti*, on the other hand, measure 0·400–0·451 (0·425 mm. × 0·008 mm. after the same treatment (clearing slowly in glycerine).

SUMMARY

Dipetalonema sprenti n.sp. is described from specimens removed from the peritoneal cavity of beavers (*Castor canadensis*) from Ontario, Canada. *D. sprenti* is found to be similar to *D. arbuta* Highby, 1943, but differs in the larger size of the eggs and fully developed embryos, the greater lengths of the spicules, the arrangement of the medial post-anal papillae, the more posterior position of the nerve ring, the greater length of the tail and in the considerably larger size of the body. *D. sprenti* is the fourth species of *Dipetalonema* described from rodents.

The writer is grateful to Dr H. B. Speakman, Director of the Ontario Research Foundation, and Dr A. M. Fallis, Director of the Department of Parasitology, for their advice and encouragement. He also wishes to express his gratitude to Dr E. W. Price, Assistant Chief, Zoological Division, United States Bureau of Animal Industry, for permitting him to examine the type material of *D. arbuta*. The writer is pleased to acknowledge the financial assistance of the National Research Council and the Research Council of Ontario. He is particularly grateful to those who collected the specimens studied herein, particularly to Miss B. M. Caldwell of the Ontario Department of Lands and Forests, and Mr Ted Cusson, Wildlife Management Officer at North Bay, Ontario, who collected the first male specimens of *D. sprenti*. The writer acknowledges his gratitude to Dr Everett E. Wehr of the Zoological Division, United States Bureau of Animal Industry, for critically reading the manuscript of this paper.

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Key to lettering on figures

a	anus	*ls*	left spicule
am	amphid	*oes*	oesophagus
bw	body wall	*p*	papilla
c	cuticle	*ph*	phasmid
e	embryo	*rs*	right spicule
i	intestine	*u*	uterus
la	lateral appendage	*va*	vagina
ld	left deirid	*v*	vulva

(*MS. received for publication* 11. v. 1953.—Ed.)

ADDENDUM

The microfilariae of **Dipetalonema sprenti** n.sp.

(With Figure 13)

Since the MS. of the foregoing paper was submitted for publication the writer obtained, through the courtesy of Mr C. J. Whitelaw of the Ontario Department of Lands and Forests, a female beaver from Algonquin Park, Ontario (11 June 1953). Three female and two male specimens of *D. sprenti* n.sp. were found in the coelom of this beaver. Microfilariae were found, moreover, in blood from the heart. As it is highly desirable that microfilariae be described with adult forms whenever possible, the following is the description of the microfilaria found in this beaver.

Table 2. *The measurements* of microfilariae of* Dipetalonema sprenti *n.sp.*

No. ...	1	2	3	4	5	6	7	8	9	10	Average
Length	344	401	399	425	322	334	336	416	409	450	387
Ceph. sp.	3·8	3·3	4·0	3·1	3·8	3·6	3·8	3·5	3·9	3·1	3·6
N.R.	22·1	19·7	20·5	18·6	19·6	18·3	17·0	20·6	20·0	15·7	19·2
Ex.p.	29·8	27·0	28·5	24·8	28·7	26·4	—	28·2	25·8	26·3	27·3
I.B. I.	45·0	43·3	47·7	40·3	45·4	44·8	42·5	44·1	45·1	41·7	44·0
I.B. II.	60·2	62·5	60·9	—	59·8	60·0	58·1	—	61·3	56·8	60·0
R.-I.	64·2	65·1	66·2	64·6	—	65·0	63·2	65·1	64·5	59·9	64·2
A.P.	75·8	78·9	78·8	77·0	73·7	77·6	77·2	77·8	78·7	76·3	77·2
L.T.C.	90·2	91·4	90·0	90·6	89·5	89·2	91·1	91·7	90·3	90·7	90·3

* The total length is given in microns; the fixed points are expressed as percentages of the total length. Abbreviations are as follows: *Ceph. sp.* = cephalic space; *N.R.* = nerve ring; *Ex.p.* = excretory pore; *I.B.I.* = anterior margin of inner body; *I.B. II* = posterior margin of inner body; *R.-I.* = first rectal ('genital') cell; *A.P.* = anal pore; *L.T.C.* = last tail cell.

Description. (Twelve specimens, Giemsa-stained.) The length and positions of the various fixed points are given in Table 2, and Fig. 13 is an illustration of specimen no. 3. A hyaline sheath can be seen on three of the twelve specimens studied. The sheath is extremely delicate and closely applied to the body of the worm. The microfilaria is wide and blunt at the anterior end but the tail end tapers to a fine point behind the anal pore. The cephalic space is longer than it is wide. The nuclear column terminates anteriorly with a pair of nuclei which are often somewhat isolated from the remaining nuclei. The nuclear column behind these cells consists of about three rows of more or less round nuclei, although as many as four rows are sometimes present in the thicker parts of the worm. Two to three rows of large nuclei occur immediately behind the anal pore; these have a crowded appearance. Farther back in the tail a single row of large scattered nuclei occur. Two elongate nuclei are present in the thinner part of the tail, and these remain isolated by several microns from those preceding them. The nerve ring occurs as a prominent break in the nuclear column. The nucleus of the excretory cell, occurring about 11–12 μ behind the excretory pore, is about half as wide as the body in that region. An inner body, although present, is ill-defined in the specimens studied,

appearing as a diffuse reddish body in front of the rectal cell. The first rectal cell is large, its nucleus occupying three-quarters of the width of the body in that region.

Host. Castor canadensis Kuhl.

Location. Blood.

relative positions of the various fixed points. Highby (1943) reported the microfilariae of *D. arbuta* as being unsheathed. The writer has re-examined specimens of *Mf. arbuta* which he has collected from porcupines in Ontario. All these specimens lack sheathes. If a sheath is a constant

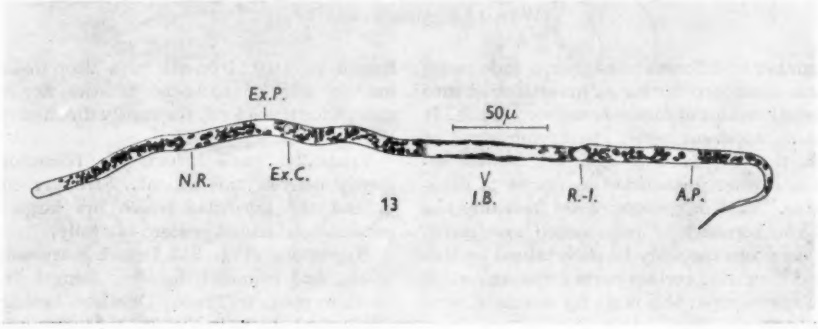


Fig. 13. *Dipetalonema sprenti* n.sp. Side view of microfilaria (specimen no. 3). Sheath not apparent. For abbreviations, see Table 2. Drawn with aid of camera lucida.

Locality. Lake Sasajewan, Algonquin Park, Ontario, Canada.

Specimens. Zoological Division, United States Bureau of Animal Industry (specimens 1–4).

The microfilariae of *D. sprenti* n.sp. are markedly larger than those of *D. arbuta*, although they are otherwise similar in nuclear detail and in the feature of *Mf. sprenti*, even though it may not be visible on some specimens because of its extreme delicacy, then the presence or absence of a sheath may be an important distinguishing character between *Mf. sprenti* and *Mf. arbuta*.

29 June 1953. ROY C. ANDERSON

THE SYSTEMATIC STATUS OF *IXODES PERCAVATUS* VAR. *ROTHSCHILDI* NUTTALL & WARBURTON, 1911

By D. R. ARTHUR, *Department of Zoology, King's College, University of London*

(With 12 Figures in the Text)

A large number of different ticks from a wide range of hosts was examined during an investigation into the host relationships of *Ixodes hexagonus* Leach. It soon became apparent when identifying some of these ticks that the existing descriptions were inadequate and often inaccurate in points of diagnostic value. Most of the errors are probably the result of the scrutiny of unmounted specimens. Detailed structure can only be ascertained by dissecting and mounting certain parts for examination under the microscope: this is so, for example, with the hypostome and tarsi.

From existing descriptions I found it difficult to identify the nymphs of *I. percavatus* var. *rothschildi* Nuttall & Warburton, 1911; identification being possible only after seeing the type collection at the British Museum (Natural History). Two females and three nymphs removed from a puffin (ex. Rothschild collection—no locality or date cited) constitute this collection. The present descriptions are based on this material, supplemented by ten females and five nymphs from a dead herring gull (*Larus argentatus*) Perranporth, N.W. Cornwall, 15.6.1912 (ex. G. H. F. Nuttall collection, no. 1736); three nymphs collected from a Manx shearwater (*Puffinus p. puffinus*), Skokholm Is., 1950 (ex. G. B. Thompson private collection).

FEMALE

Capitulum (Figs. 1 and 2). Length, 0·62 mm.; width of basis across the cornua, 0·55 mm. Basis capituli, dorsal surface mildly convex, smooth, shining, impunctate and lacking hairs. Posterior margin straight or slightly sinuous, salient. Porose areas oval, large, extending back almost to the dorsal ridge and forward almost to the base of the palp; separated from each other by slightly less than their maximum width. Cornua distinct, strong, broad, rounded apically and elevated over the salient posterior edge. Palps broad, short, rounded apically; no distinct suture line visible between segments 2 and 3; median margins gently curved, lateral profile straight, but having a pronounced indentation basally. Combined length of segments II and III, 0·38 mm.; greatest breadth, 0·15 mm. Palpal segment I broader than long, breadth to length as 1·10 : 1·0; set in a deep fossa on the anterior edge of the basis. Bristles, few and short, except for three long, forwardly directed bristles on the apex of segment III.

Ventrally, basis broad, the posterior margin gently convex and salient. Strongly constricted behind the auriculae which are large, rounded extensions directed postero-laterally.

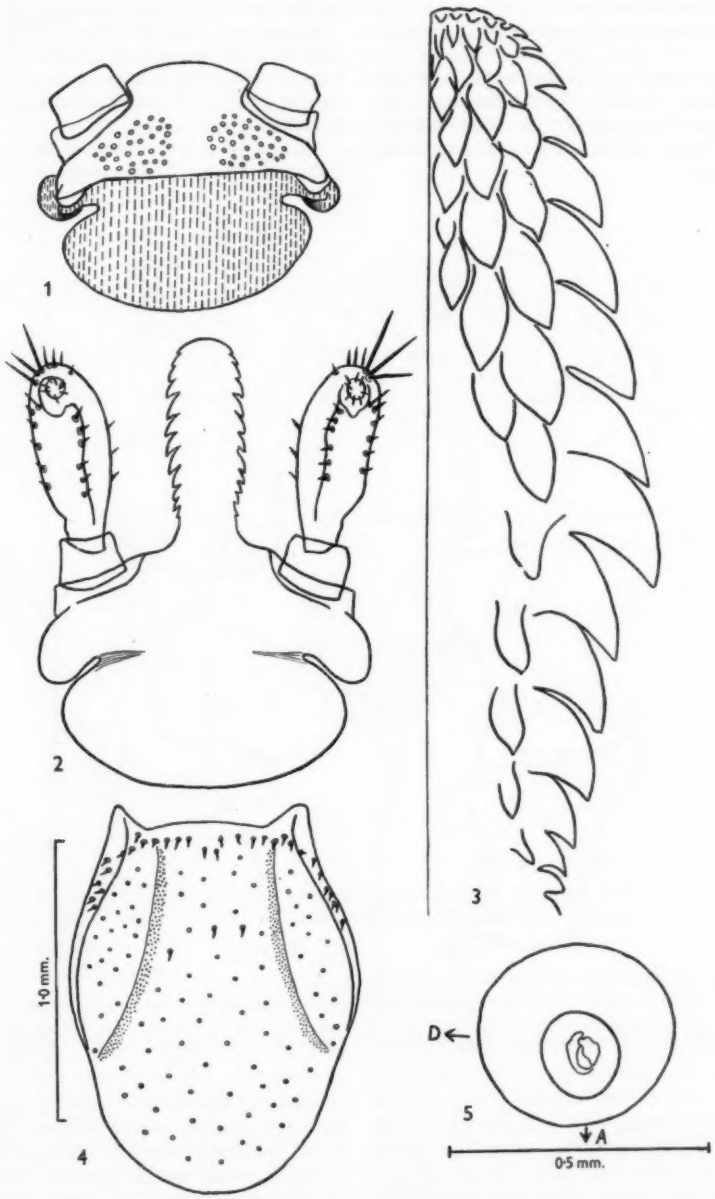
Hypostome (Fig. 3). Broad, narrowing proximally, and rounded distally. Length from basal tooth to apex, 0·37 mm. Denticles arranged 5/5 to 2/2 to the base, as in Fig. 3, 14–15 prominent lateral denticles; proximal median unarmed area.

Scutum (Fig. 4). Length, 1·42 mm.; breadth, 1·06 mm. Suboval, longer than wide, widest in front of the middle, rounded behind. Cervical grooves as valleys separating the median and lateral areas, converging slightly at first and then diverging; extending almost to the postero-lateral margins. Median area particularly convex. Lateral carinae present. Punctations fine, but rugose areas present in lateral field. Bristles, few, short and broad. Scapulae pointed and emargination well defined.

Legs. Long and slender, surface smooth and shining. Tarsi long and ending in a slight hump (Fig. 7). Length of tarsus I, 0·76 mm.; metatarsus, 0·52 mm. Length of tarsus IV, 0·69 mm.; length of metatarsus IV, 0·57 mm. Bristles on tarsi vary in length but all are finely pointed and delicate; on other segments bristles broader, stronger, frequently multispinose.

Coxae (Fig. 6). Mildly convex, smooth, shining, with few punctations. Circle of short bristles around the distal edge, longer bristles at the base of the spurs and on the posterior margin of the coxa. Internal spur on coxa I drawn out into a point, those on coxae II and III of about equal size, that on coxa IV reduced to a salient projection. External spurs present on all coxae, that on coxa I being somewhat recurved and rounded distally, those on coxae II–IV tapering and of about equal size. Trochanter spurs present on legs I–III.

Spiracular plate (Fig. 5). Surface slightly concave, a little elevated over the surface of the body. Round, with macula eccentric on the antero-ventral side. Goblets numerous.



Figs. 1–5. *I. rothschildi*. Fig. 1. Female. Basis capituli, dorsal view. Fig. 2. Female. Capitulum, ventral view. Fig. 3. Female. One half of the hypostome drawn from the ventral side. Fig. 4. Female. Scutum. Fig. 5. Female. Spiracle. *A*, anterior; *D*, dorsal.

Genital aperture. Between coxae III, genital grooves straight and divergent.

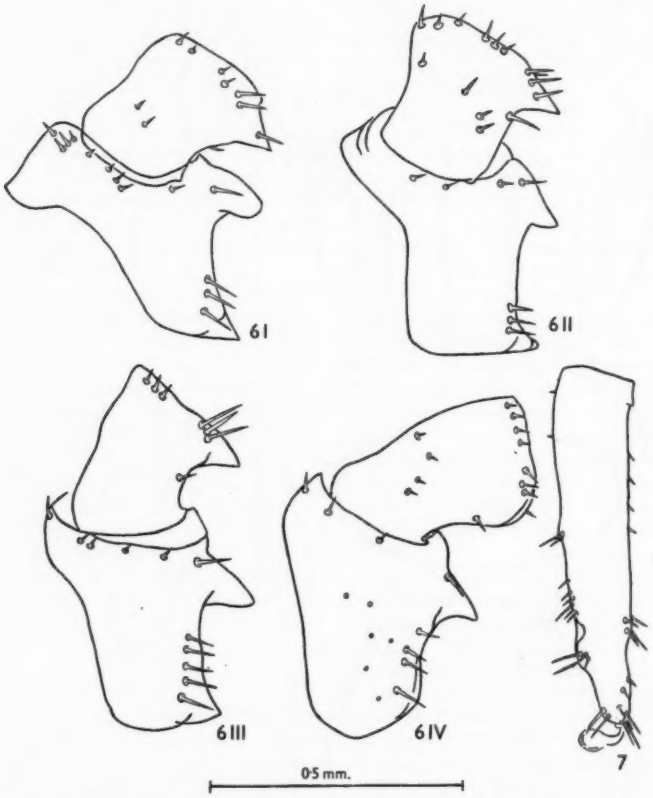
Anal groove. Parallel sided, rounded in front of the anus.

Haller's organ (Fig. 8). Long, anterior trough shallow; posterior capsule open, elongate oval, deeper anteriorly than posteriorly. Seven sensilla present, all of which are curved forward and of the 'sichelhaar' type.

middle, rounded and salient behind. Auriculae, if present, reduced to divergent triangular projections.

Hypostome. Length from basal teeth to tip, 0·22 mm. Denticles arranged from base to apex as 1 row 1/1, 6 rows 2/2, 2 rows 3/3, 5 rows 4/4. Gently curved profile, 14 lateral denticles visible. Apex almost flat.

Scutum (Fig. 11). Length, 0·64 mm.; breadth, 0·47 mm. Surface finely punctate. Slightly broader



Figs. 6, 7. *I. rothschildi.* Fig. 6. Female. Coxae I–IV. Fig. 7. Female. Tarsus I.

NYMPH

Capitulum (Figs. 9 and 10). Length, from dorsal ridge to tip of hypostome, 0·37 mm.; breadth across the dorsal ridge, 0·32 mm. Basis mildly convex; smooth, impunctate. Palps narrow gradually to the base, greatest width is well forward of the combined second and third articles. Combined length of segments II and III, 0·23 mm. Dorsal ridge straight and produced laterally into two horn-like projections. Ventrally, basis broad, waisted at the

in front of the middle, becoming gently concave behind, rounded posterior margin. Cervical grooves broad, fading out before reaching postero-lateral margins, lateral carinae present. Scapulae bluntly rounded, emargination slight. Bristles short, limited to the front margin and outside the lateral carinae.

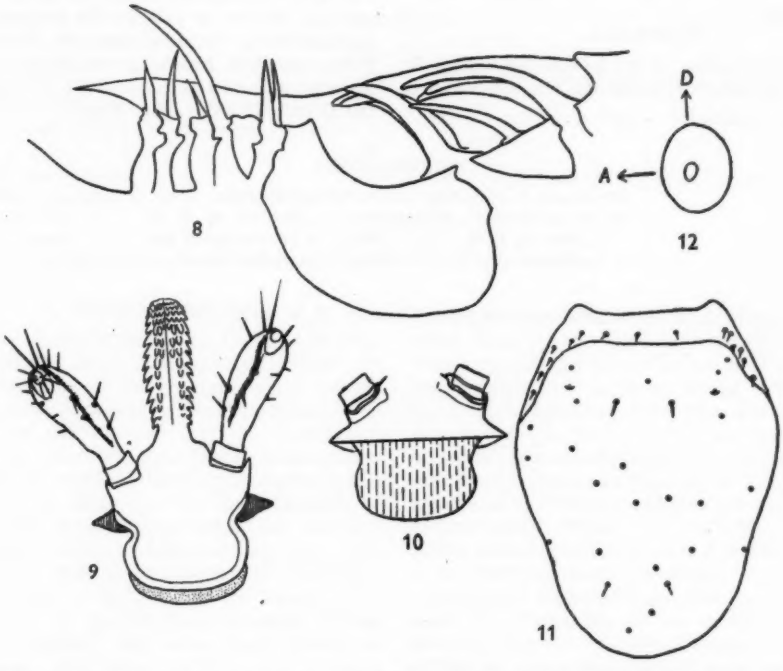
Spiracular plate (Fig. 12). Oval with long axis transverse, macula eccentric, goblets relatively few.

HOSTS

The available records in the British Museum (Natural History) show that females and nymphs of this species have been collected from a puffin (no other data) and from a dead herring gull, Perranporth, N.W. Cornwall. I now have three nymphs from a Manx shearwater at Skokholm, Pembrokeshire, in 1950.

structure is not in accord with their view. Differences are also evident in the form of the coxae as seen by comparing Fig. 6 of the present paper with those of Neumann 1906, which were reproduced by Nuttall *et al.* 1911 (Fig. 21).

For comparative purposes the three collections of *I. percavatus* in the British Museum were re-examined. The type material contains four dried,



Figs. 8–12. *I. rothschildi*. Fig. 8. Female. Haller's organ. Fig. 9. Nymph. Capitulum, ventral view. Fig. 10. Nymph. Basis capituli, dorsal view. Fig. 11. Nymph. Scutum. Fig. 12. Nymph. Spiracle.

DISCUSSION

The short description of this so-called variety by Nuttall & Warburton (in Nuttall, Warburton, Cooper & Robinson, 1911) compared it with the type *Ixodes percavatus* Neumann, 1906. Comparison of Figs. 4 and 5 by Neumann (1906) and those in the present paper reveal distinct differences in the shape of the capitulum, particularly in the postero-ventral region, and of the shape of the auriculae. The hypostome of the type (not figured) was described as 'narrow, rounded distally, 4/4 equal teeth'. Nuttall & Warburton (*loc. cit.*) drew no distinction between the hypostome of the variety and the type: presumably they considered them to be similar. The present description (p. 222) of this fully-fed females from Nightingale Island, Tristan da Cunha (west of the Cape of Good Hope). Three of the four specimens are labelled 'types named by L. G. Neumann, 1905'. This material is badly mutilated; all the specimens are without hypostomes, palpal segments II–IV are missing in all except one, and the first tarsal segment is invariably absent. For examination these specimens were treated with 0·5% sodium phosphate (tri-basic), and then it was seen that the body was covered with moderately long bristles which are longer than those of the variety. The available characters of the specimens in this collection show that *I. percavatus* is synonymous with *I. auritulus* Neumann, 1904.

The second collection from the penguin, *Eudyp-*

tula minor from Perth, West Australia (N. 1850= Cleland 17) contains two females which agree with the so-called variety.

The three females from the third collection removed from a Dove Petrel, Stephen Island, 1922 (ex. R. J. Tillyard, N. 3862) are unquestionably *Ixodes auritulus*. I am, therefore, of the opinion that *I. percavatus* is a non-valid species and should be sunk in synonymy, and the variety created by Nuttall & Warburton raised to the specific rank of *I. rothschildi*.

SUMMARY

1. The description of the female and nymph of the so-called variety *I. percavatus* var. *rothschildi* is enlarged.

2. As far as the evidence is available it parasitizes marine birds and is probably a three-host tick.

3. Its systematic affinities are discussed, and since *I. percavatus* is synonymous with *I. auritulus*, then the variety of Nuttall & Warburton should be raised to the specific rank of *I. rothschildi*.

In establishing the synonymy of *I. percavatus*, I received considerable help from Mr E. Browning, and am grateful to him for his patience and forbearance of my continual demands. Both Dr Owen Evans and Mr E. Browning very kindly checked the description, and the material is now deposited in the British Museum (Nat. Hist.).

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THE HOST RELATIONSHIPS OF *IXODES HEXAGONUS* LEACH IN BRITAIN

By D. R. ARTHUR, *Department of Zoology, King's College, University of London*

(With 3 Figures in the Text)

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I. INTRODUCTION

Knowledge of *Ixodes hexagonus* Leach is so fragmentary that it seems justifiable to present the following sparse notes from an exploratory investigation of certain ecological aspects. The presentation will at least indicate the kind of information required. The field work of the present investigation was carried out mainly in 1949–51. Throughout this paper the term 'nests' covers such habitats as burrows, setts, dens, etc.; the term 'tick activity' refers to the infestation of the host and not to the physiological condition of the tick, so that when ticks are said to be 'active' it means that they occur on the host in some numbers. Unless otherwise indicated, the word 'tick' refers to *I. hexagonus*.

Nuttall (1911) and Schulze (1941) stated that the free-living stages of *I. hexagonus* are found in the nests of the hosts. The seasonal activity of the different stages is consequently difficult to determine because the nests of some hosts (e.g. foxes, badgers) are inaccessible, or because the number of nests (e.g. hedgehogs) found in a workable area is inadequate to arrive at precise conclusions. Counts of *I. hexagonus* on wild animals can only give a blurred picture of seasonal activity, for the numbers of ticks on the hosts may be influenced by such factors as delayed exposure to infestation, sexual behaviour or other habits of hosts.

So far I have only attempted to determine the seasonal occurrence of ticks on their habitual hosts. Material for this purpose has been obtained by shooting and trapping representative samples of vertebrates from selected areas in south Wales and south-east England. Foxes, badgers, rabbits and hares were examined in the field immediately after being shot. Stoats, weasels, rats, voles and mice were trapped and placed in paper or fine muslin bags, which were securely sealed and examined 24 hr. later. Hedgehogs were placed alive in biscuit tins, brought to the laboratory and transferred to breeding cages suspended over water-baths. Where samples were adequate throughout the year, it was possible to determine whether a host was 'habitual' or 'accidental'. Milne (1949) has indicated the difficulties attached to the study of *I. ricinus* L. on wild hosts. Much the same difficulties occur in the case of *I. hexagonus*. Different host-species require different methods of capture; all the methods are time-absorbing and subject to the fluctuations of chance, so that to a very large extent hosts were trapped indiscriminately in an endeavour to get adequate samples. In the body of this paper the common names of animals are used as given in the British Museum list of British vertebrates (1935). The corresponding scientific names are shown in Table 1, together with the total numbers of specimens examined and the numbers infested over a period of 2 years.

The distribution of *I. hexagonus* in Britain was ascertained from the literature and from correspondents who supplied type material for checking. The greater part of the results, however, came from my own observations in the field. Much of the material is now retained in my own collection and has been used in elucidating the morphology of this species. The specimens from polecats are in the collection of the National Museum of Wales and those from human beings in the British Museum (Natural History).

Table 1. *List of wild animals examined during tick activity*

(The list shows total specimens, common names and specific names. Nomenclature follows the British Museum *List of British Vertebrates* (1935). The figures in brackets show total number of specimens infested with *I. hexagonus*.)

Mammals		Stages infesting hosts		
		Females	Nymphs	Larvae
116 Hedgehogs (72)	*Erinaceus europaeus*	×	×	×
32 Stoats (18)	*Mustela erminea*	×	×	×
7 Weasels (4)	*M. nivalis*	×	×	.
*15 Polecats (13)	*M. putorius*	×	×	×
*5 Otters (4)	*Lutra lutra*	×	.	.
5 Badgers (2)	*Meles meles*	×	.	.
12 Foxes (3)	*Vulpes vulpes*	×	×	×
5 Dogs (1)	*Canis familiaris*	×	×	×
13 Grey squirrels (0)	*Sciurus leucoris*	.	.	.
8 Red squirrels (0)	*S. vulgaris*	.	.	.
34 Moles (1)	*Talpa europea*	×	.	.
18 Common shrews (0)	*Sorex araneus*	.	.	.
183 Rabbits (0)	*Oryctolagus cuniculus*	.	.	.
19 Hares (0)	*Lepus europaeus*	.	.	.
23 Bank voles (0)	*Clethrionomys glareolus*	.	.	.
28 Short-tailed voles (0)	*Microtus agrestis*	.	.	.
35 Long-tailed field mice (0)	*Apodemus sylvaticus*	.	.	.
27 Brown rats (1)	*Rattus norvegicus*	×	.	.
14 Cats (1)	*Felis domesticus*	×	.	.
20 Cattle (1)	*Bos bovis*	×	.	.
20 Sheep (0)	*Ovis aries*	.	.	.
*29 Men (29)		×	×	×
5 Ferrets (2)	*Mustela putorius* (domesticated variety)	×	×	×

* Not specifically recorded but data obtained from various sources.

Dr A. Milne very kindly permitted me to use his unpublished data on *I. hexagonus* from northern England, and I am indebted to both him and Dr P. Tate for their comments on the typescript. Mr E. Browning of the British Museum gave me access to the museum records of *I. hexagonus*, and both he and Dr G. Owen-Evans have criticized the manuscript. Dr F. A. Turk supplied me with information about the ticks of Cornwall and the Scilly Isles. In addition, I received ticks and/or infested hosts from Mr G. B. Thompson, Cambridge; Mr W. Thomas, Ammanford; Mr M. Froud, M.Sc., Wye; Mr J. Muggeridge, Isle of Grain; Mr G. Saunders, Gavestone; Mr J. Ash, Silwood Park, Berks; Mr Colin Matheson, M.A., Cardiff, and Mr E. Neale, Taunton. To all these I am deeply indebted, as well as to those who supplied occasional material.

II. SEASONAL ACTIVITY OF *IXODES HEXAGONUS*

At the outset it should be emphasized that the conclusions regarding the seasonal activity from somewhat inadequate data covering only 2 years must be viewed with some reserve. In interpreting the data the following important questions arise: (*a*) What numerical differences in the monthly averages are biologically significant, i.e. what portions of the differences are due to an actual uneven monthly distribution and what to sampling errors? Small samples may reveal only major differences as statistically significant. (*b*) Are the number of hosts examined giving a representative picture of the changes which are going on in time or are there important events being missed by inadequacy of host material and, further, is it safe to assume that seasonal activity is the same in south Wales and south-east England? Paucity of material forces us to form a picture of activity on the basis of monthly infestations for both regions combined.

The data for seasonal activity have been based mainly on the occurrence of the ticks on hedgehogs, stoats and weasels. In each case a total count of all females, nymphs and larvae on the body of each individual was made. The degree of tick infestation on the various regions of the body varies considerably; larvae are found on the face and tips of the ears as also are the nymphs, but the latter are sometimes found in association with the females in the axillae of the forelegs, in the groin and in the perineal regions; the flanks and back are infrequent sites for parasitization by *I. hexagonus*.

The data for hedgehogs for 1948–9 (Fig. 1) show that April and May were the periods of heaviest

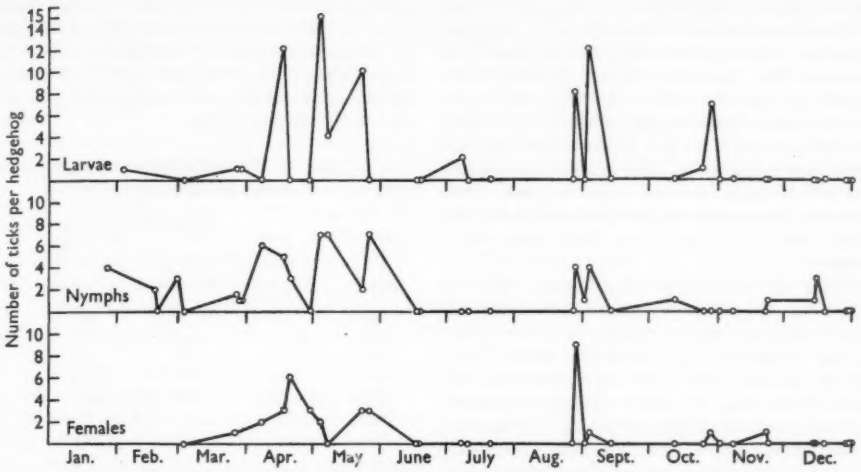

Fig. 1. The seasonal occurrence of *I. hexagonus* on hedgehogs in 1948–1949 (observations made in south Wales and south-east England).

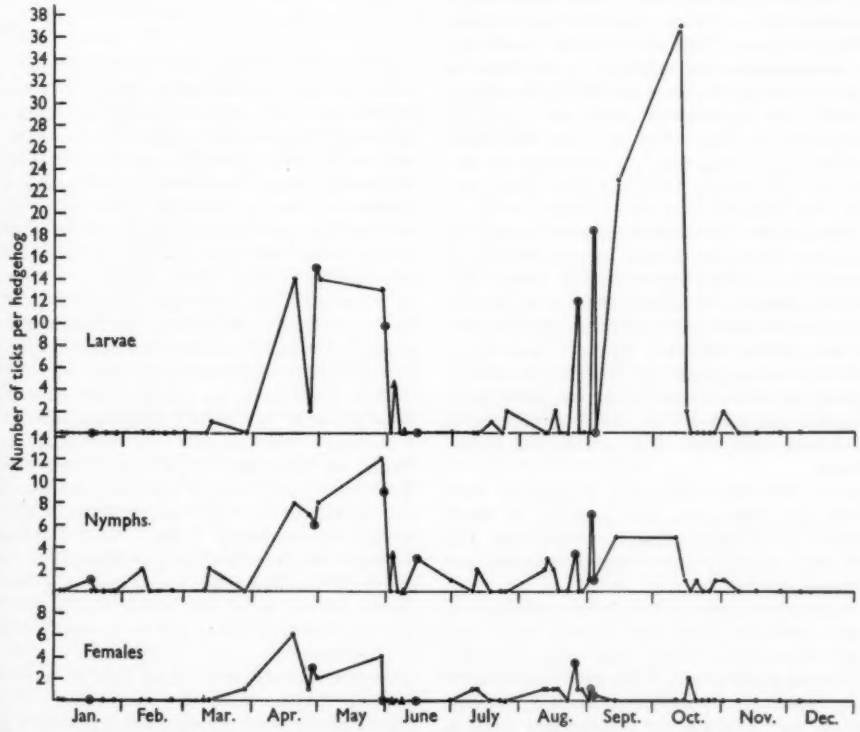

Fig. 2. The seasonal occurrence of *I. hexagonus* on hedgehogs in 1950 (observations made in south Wales and south-east England). ⊙, mean values of tick populations based on two hedgehogs examined on the same day; ▲, mean values of tick populations based on three hedgehogs examined on the same day.

infestation. This was followed by a decline in June and July with a renewal of activity in late August which persisted with fluctuations until October. The 1950 results (Fig. 2), based on nearly double the number of samples for 1948–9, show a similar bimodal occurrence, with a spring peak in April–May and an autumn peak in late August–October. Of five hedgehogs trapped by Milne (unpublished data) in northern England none was infested with *I. hexagonus*, although two of the five were examined when this tick is active in south Wales and south-east England.

Practical difficulties arise in assessing the true 'ground activity' of *I. hexagonus*, i.e. temporal changes in numbers ready to feed given the opportunity. *I. hexagonus* most probably occurs in or around the nests of the hosts and, assuming the stability of the 'home', hosts should constantly return to the source of infestation. Under natural conditions, however, various edaphic and biotic factors may from time to time cause hedgehogs to abandon their nests and construct new ones. The attached ticks on completing engorgement on such animals will drop off and for a time these hosts should remain free of ticks. Without more exact knowledge of these host movements, however, further speculation is unprofitable. Again there is the question of adult hedgehogs sharing the available unfed tick population with their young. Hedgehogs pair in April–May and the first litter (3–6) is born May–June, which is probably too late to affect the numbers of ticks per host. They pair again in July–August and a second litter arrives in August–September. Since ticks attach themselves to the parent host, presumably young hedgehogs are not neglected. The available active ticks in the nest would therefore be distributed among five to eight bodies after birth, as compared with two before, and a false picture of their 'ground' activity is obtained by determining the occurrence of the parasite on the host. Clearly then, the problem of assessing the seasonal activity of *I. hexagonus* is quite different from that of *I. ricinus* (see Milne, 1947, 1949).

Between 1948 and 1951, 116 hedgehogs were examined for ticks, and the number of hosts parasitized by different stages ascertained. The numbers and monthly percentage infestation are summarized in Table 2.

Larval-infested animals were first collected in February and were most numerous in April and May, and less so later in the year. Nymphs were present during each month of the year; the number of infested animals increased from January to May, declined to July and rose to a secondary peak in September. Hedgehogs infested with female ticks were first collected in March, reached a peak in April and, after a drop to June, attained a secondary peak in the last fortnight of August. Animals parasitized with nymphs were found more frequently than those by larvae and females. Larval- and female-infested animals were found in about equal numbers and no certain explanation can be offered for this, unless it be inadequate sampling.

Table 2. *The infestation of hedgehogs by* Ixodes hexagonus (1948–51)

Month	No. trapped	% infestation with one or more tick stages		
		Females	Nymphs	Larvae
Jan.	10	—	20	—
Feb.	9	—	33	22
Mar.	11	27	55	36
Apr.	9	100	88	67
May	7	57	100	71
June	13	—	38	15
July	9	22	11	43
Aug.	11	73	54	27
Sept.	10	40	80	40
Oct.	10	20	50	40
Nov.	8	13	26	13
Dec.	9	—	33	—
	116			

Thirty-two stoats and seven weasels were examined and the indications from the present data are that ticks are active on these hosts in spring and autumn. The greatest number of larvae are active later in the spring than the nymphs and adults, but the three peaks coincided in the autumn. The number of young in the stoat litter varies from 5 to 10 (breeding period April–May), so that, assuming no death-rate and that ticks are found in the nest, the number of ticks active will now be distributed among more hosts, thus reducing the population of the parasites per individual. The same is true for weasels: the usual number in a litter is four to six, and there may be two or three litters in the year.

Five stoats and two weasels were examined for ticks by Milne in northern England; one nymph of *I. hexagonus* was collected from one weasel (Lordenshaws, 23 June 1944), the others being free of ticks. The location and dates of collection of these samples were as follows: weasels, Lordenshaws, 9 April 1943; stoats, Lordenshaws, 7 May 1943; Lordenshaws, 21 May 1943; Lordenshaws, 28 April 1944; Lordenshaws, 13 October 1944; Lordenshaws, 1 June 1945. Most of these dates fall within the period of tick activity in south Wales and south-east England.

Unfortunately, the information on the third mustelid, the polecat, refers only to tick-infested animals. Apart from the record for Pantygoitre Farm, all the other information is probably qualitative. Accordingly, no reliance can be placed on this data in assessing the activity of the tick. It is of interest, however, to note that four polecats were

infested in January and February (1949–50), one in late November and one in early December.

The number of larger hosts are inadequate to formulate precise conclusions: of 12 foxes, only 3 were infested (8 April, 12 May, 30 March). Milne's unpublished data show a similar feature and only 1 female tick was collected from 24 adults, while 2 cubs out of 31 yielded 8 females, 24 nymphs and 3 larvae of *I. hexagonus*. In general, his findings (unpublished data) show that *I. ricinus* is a little more plentiful than *I. canisuga* and much more plentiful than *I. hexagonus* on foxes.

III. REVIEW AND DISCUSSION OF PREVIOUS HOST RECORDS

Most records of the hosts of *I. hexagonus* are to be found in the following works: Neumann (1901); Nuttall, Warburton, Cooper & Robinson (1911); Nuttall (1915); Hirst (1916); Walton (1918); Riley (1921); Schulze & Schlottke (1929); Oswald (1939); Thompson (1934, 1935, 1936, 1937); Elton (1937); Senevet (1937); Browning & Airy-Shaw (1944); Turk (1945); Evans (1951); and Milne (unpublished data). Some of these authors do not state the stages of the tick found on the hosts, though others do; the following host-list, compiled from the above sources, takes no account of the stage of the tick found infesting the host: (1) hedgehog; (2) stoat; (3) weasel; (4) polecat; (5) Irish stoat, probably a subspecies of the stoat; (6) ferret; (7) fox; (8) otter; (9) rabbit; (10) dog; (11) cat; (12) cow; (13) goat; (14) mink; (15) sable; (16) red squirrel; (17) grey squirrel; (18) pine marten; (19) beech marten; (20) hare; (21) marmot; (22) opossum (Browning & Airy-Shaw, 1944), *Didelphys* (Senevet, 1937); (23) *Spermophilus* sp.; (24) wolves; (25) porcupine (Browning & Airy-Shaw, 1944), *Hystrix cristata* (Senevet, 1937); (26) rat; (27) badger; (28) American badger; (29) tarabagan; (30) sheep; (31) man; (32) sand martin; (33) corncrake; (34) golden plover; (35) British blue tit; (36) wren; (37) horse; (38) coypu rat.

Some of these records are American in origin: females were collected from squirrel, cat, sheep, rabbit (north America) and from the coypu rat in South America (Nuttall *et al.* 1911); unspecified stages from weasels (Nuttall, 1915) and from foxes in Canada (Riley, 1921). Other hosts given in the list are indigenous to America: 'opossums (Didelphyidae) range from the United States to the Argentine' (Tate-Regan, 1946), the woodchuck (*Marmota monax*) and the American badger (*Meles taxus*) are both North American species. *Ixodes hexagonus* does not occur in the New World (Cooley & Kohls, 1945; Bequaert, 1946), so that the records of this tick on American hosts are invalid. Similarly, the occurrence of *I. hexagonus* var. *cookei* (=*I. cookei* Packard) in Yugoslavia (Oswald, 1939) is doubtful, a view to which Kohls (private communication, 3 February 1947) subscribes.

Frequently specific names of hosts are lacking (e.g. mink, sable, wolf, marmot) and this may cause confusion, for different animals known only by such common names may occur in central Europe, Asia and/or North America. Without more exact knowledge of the host species and its distribution these records of *I. hexagonus* must remain questionable.

Two records of *I. hexagonus* given by Thompson (1937) have now been amended: the specimens from the blue tit are *I. passericola* Sch. and those from the wren are *Ixodes* sp. (? *dryadis* Sch. & Schl., 1929).

Nos. 21–3 and 28 can be eliminated from the host-list because they are indigenous to the Americas or else the record is from America; nos. 15 and 24 because no specific names are cited and the distribution of the hosts is unknown; nos. 35 and 36 because of incorrect identification. Since we are concerned with the British hosts of this tick, we can also disregard nos. 5, 15, 19, 24, 25 and 29.

Only *I. trianguliceps* Birula has been found on the bodies and in the nests of mice, voles and shrews (Elton, Ford, Baker & Gardner, 1931; Elton, 1934; Davis, 1934; Hora, 1934; Elton & Keay, 1936), but the absence of *I. hexagonus* cannot be considered significant, because we have no knowledge as to whether these ticks occur on other hosts in these localities.

IV. PRESENT HOST RECORDS

When investigating the host relationships of *I. hexagonus*, other species of tick were often encountered. A hedgehog taken in Kent carried one nymph of *Haemaphysalis cinnabarina* var. *punctata* Can & Fanz.; *Ixodes trianguliceps* Birula was found on small mammals, *I. melicola* Schl. on a badger, *I. canisuga* on dogs and foxes, *I. rothschildi* (=*I. percavatus rothschildi* Nuttall & Warburton, 1911), *I. frontalis* Panzer, *I. passericola* P.Schl., *Ceratixodes uriae* White and *Haemaphysalis cinnabarina* var. *punctata* on birds.

(*a*) *Hosts to one or more stages of* Ixodes hexagonus

In south Wales and south-east England the following animals are hosts to one or more stages of *I. hexagonus*: (1) hedgehog, (2) weasel, (3) stoat, (4) polecat, (5) ferret, (6) fox, (7) otter, (8) dog, (9) cat, (10) cow, (11) rat, (12) mole, (13) badger, (14) man, (15) escaped coypu (given in British Museum material, but without date or place).

For comparative purposes the known mammalian hosts of *I. hexagonus* in northern England and south Wales and south-east England have been tabulated

(Table 3). The northern England data have been compiled from the unpublished records of Milne and from his published data on *I. ricinus* (Milne, 1949). The results indicate that more mammals were parasitized with *I. hexagonus* in the southern region of the country, but a comparison of the degree of infestation of animals from the two areas cannot be really sound. Ticks were found only on three hosts (foxes, otters and weasels) common to both areas; except for foxes the numbers of otters and weasels are inadequate to pronounce judgement. In any case, only tick-infested otters were reported from the southern region, which invalidates their mean values, for obviously if there are uninfested hosts like hedgehogs, the same is true for otters. Milne reported his results for fifty-six foxes under adults and cubs, and comparison of the means with those from the south (twelve examined) are shown below.

Table 3. *Comparison of total infestations of* Ixodes hexagonus *on mammals in northern England and in south Wales and south-east England**

(The figures in parentheses refer to total infested specimens.)

	North England				South Wales and south-east England			
Host	No. examined	Females	Nymphs	Larvae	No. examined	Females	Nymphs	Larvae
Common moles (*Talpa europaea*)	17 (0)	.	.	.	34 (1)	1	0	0
Shrews (*Sorex araneus*)	42 (0)	.	.	.	18 (0)	.	.	.
Hedgehogs (*Erinaceus europaeus*)	5 (0)	.	.	.	116 (72)	79	226	313
Foxes (*Vulpes vulpes*) adults	25 (1)	1	0	0	10 (3)	23	59	41
Foxes (*V. vulpes*), cubs	31 (2)	8	24	3	2 (0)	.	.	.
Badgers (*Meles meles*)	9 (0)	.	.	.	5 (2)	3	2	0
Otters (*Lutra lutra*)	2 (1)	1	3	0	5 (5)	0	5	3
Stoats (*Mustela erminea*)	5 (0)	.	.	.	32 (18)	43	56	94
Weasels (*M. nivalis*)	2 (1)	0	1	0	7 (4)	7	13	0
Rabbits (*Oryctolagus cuniculus*)	790 (2)	1	0	1	183 (0)	.	.	.
Hares (*Lepus europaeus*)	21 (0)	.	.	.	19 (0)	.	.	.
Bank voles (*Clethrionomys glareolus*)	2 (0)	.	.	.	23 (0)	.	.	.
Short-tailed voles (*Microtus agrestis*)	23 (0)	.	.	.	28 (0)	.	.	.
Field mice (*Apodemus sylvaticus*)	17 (0)	.	.	.	35 (0)	.	.	.
Brown rats (*Rattus norvegicus*)	5 (0)	.	.	.	27 (1)	1	0	0
Red squirrel (*Sciurus vulgaris*)	4 (0)	.	.	.	8 (0)	.	.	.
Grey squirrel (*S. leucoris*)	.	.	.	.	13 (0)	.	.	.
Roe deer (*Capreolus capreolus*)	8 (0)	.	.	.		.	.	.
Dogs (*Canis familiaris*)	.	.	.	.	5 (5)	2	43	42
Cats (*Felis domesticus*)	.	.	.	.	14 (1)	1	0	0
Cows (*Bos bovis*)	? (0)	.	.	.	(1)	1	0	0
Sheep (*Ovis aries*)	(0)	.	.	.	35 (0)	.	.	.
Ferrets (*Mustela putorius*), domesticated variety	.	.	.	.	5 (2)	1	3	5

* The polecat has been omitted from this list because the samples are probably qualitative.

	North England			South Wales and south-east England		
	Females	Nymphs	Larvae	Females	Nymphs	Larvae
Foxes: adults	0·04	0	0	2·3	5·9	4·1
cubs	0·26	0·78	0·097	0	0	0
Adults and cubs	0·16	0·43	0·053	1·93	4·93	3·42

From this meagre information it would appear that heaviest infestations occur in the south: it is, however, far from conclusive.

I. hexagonus parasitizes at least fifteen mammals, but we have no satisfactory evidence that it occurs on birds. On the other hand, *I. ricinus* is found on at least twenty-nine species of mammals and thirty-nine bird species (Milne, 1949). The probability of *I. ricinus* having a wider range of bird hosts has been discussed (Arthur, 1952*a*) and additional British records are from the siskin (*Carduelis spinus*), the white wagtail (*Motacilla alba*), the tree pipit (*Anthus trivialis*), the sparrow hawk (*Accipiter nisus*) and the whitethroat (*Sylvia communis*) (Arthur & Thompson, in the Press).

(*b*) *Non-hosts*

Nine mammalian species did not yield ticks (Table 1), and of these red and grey squirrels, rabbits and sheep are mentioned as hosts by other writers. Normally among tick-yielding species (i.e. foxes, hedgehogs, weasels and stoats) non-infested individuals occur, so that failure to find ticks on other hosts may be due to inadequate sampling, lack of knowledge of the seasonal activity of these parasites and/or habits of the host. The failure to find ticks on grey squirrels may be due to sampling when very few ticks are active; thus 13 grey squirrels were examined on the following dates: 15 Jan. (1), 24 Jan. (1), 3 Feb. (1), 11 Sept. (2), 15 Oct. (4), 9 Nov. (2), 10 Nov. (1), 15 Nov. (1); or because of inadequate sampling as in the case of 8 grey squirrels collected on 26 Jan. (1), 9 Mar. (1), 10 Mar. (1), 12 Apr. (1), 27 Apr. (2), 5 May (1), 19 Oct. (1). No ticks were collected from 183 rabbits from south Wales and the Isle of Grain, Kent, even though the collections were fairly well distributed over a 12-month period. Some 45 % of the rabbits came from fields in the Isle of Grain, where 8 tick-infested hedgehogs were collected. Milne (unpublished data) found 1 larva (25 May 1943, at Lordenshaws) and one female (6 May 1943, at Thorneyburn) on 2 rabbits out of 790 he examined in Northumberland. I found no ticks on 19 hares, 23 bank voles, 28 short-tailed voles and 35 long-tailed field mice. Birds from Silwood Park, Berks (see Arthur, 1952*b*), Rudbaxton, Pembrokeshire and samples from various parts of Britain (see Arthur & Thompson, in the Press) were all free of *Ixodes hexagonus*. Milne (1949) examined 320 birds belonging to 34 species in the north of England, and in no instance was *I. hexagonus* found.

(*c*) *Hosts to different stages of* Ixodes hexagonus

Males. Males of *I. hexagonus* are very rare on their hosts: only three specimens existed in the British Museum (Natural History) before the discovery of a large number of this stage at Cirencester by Browning & Airy-Shaw (1944). In Britain Walton (1918) found one male on a badger, and Thompson (1934) reported another on a hedgehog. In addition, we now have the following records: one on a rabbit at Feock, Cornwall, April 1943 (Turk, private communication, 9 July 1945), one on an otter at Oldham (date not given) and one from a hedgehog at Putney in 1950. Twenty females and two males were taken from a female stoat at Yeovil, Somerset (21 July 1951). I found no other males associated with the hosts during the present survey. The occasional occurrence of males of this species on the host led Schulze (1941) to suggest that *I. ricinus gibbosus* is the result of crossing between males of *I. ricinus* and females of *I. hexagonus*. Morphologically this variety has characters of both species and because it occurs alongside *I. ricinus* in Macedonia is obviously not a separate geographical race.

Females. Hedgehogs, stoats, weasels, polecats and foxes are normally parasitized by females of *I. hexagonus*. In the available samples I found this stage on nine other mammals, viz. otter, dog, cat, cow, ferret, rat, mole, badger and man (see § V). No stage of *I. hexagonus* was found on stoats and hedgehogs in the north of England (Milne, unpublished data), which may be attributed either to insufficient sampling (5 stoats and 5 hedgehogs) or to relative scarcity of this species of tick in that area. Probably the latter explanation is true, for the hedgehog and stoat are the most common hosts to *I. hexagonus* in south Wales and south-east England. One female tick was found on 1 mole out of the 34 collected, probably because these mammals live below ground in open country and have little contact with regularly infested hosts. Of the 4 badgers examined 1 female *I. hexagonus* was found on one animal: Milne (1949) found 1 badger out of 9 infested with *I. ricinus*. Freshly killed badgers are hardly ever infested with parasites, except for a few fleas (Neale, 1948), and in a private communication (26 April 1949) Mr Neale informed me that the only ticks he has found on a badger came from an animal riddled with pellets and in poor condition. I identified these ticks as females and nymphs of *I. hexagonus*. This general absence of ticks from badgers is possibly due to the morning habit of delousing themselves (Neale, private communication, *loc. cit.*) and of the removal and changing of the bedding at regular intervals (Neale, 1948; Blackmore, 1948; Pitt, 1944). Frequent movement from 'sett' to 'sett' would appear to have little to do with this, for the badgers would transfer their 'fellow travellers' with them and thus ultimately set up a new focus of infestation.

One female tick was collected from 27 brown rats, and there are two other records (2 females on 2 rats)

in the Nuttall collection at the British Museum. Seemingly these are accidental hosts. The occurrence of *I. hexagonus* on sheep and cattle is likewise probably accidental. From the available data all the known tick-yielding mammals in Britain are also hosts to female ticks.

Nymphs and larvae. Six of the host-species, known to harbour females, have also yielded both nymphs and larvae and two, the weasel and the coypu, only nymphs. Since *I. hexagonus* is a 'nest' species, the weasel is probably very similar to the stoat and hedgehog. The absence of larvae on the weasel in the present work may possibly be due to insufficient sampling. The pine marten is given as a host by some writers, but with no details of infestation. No martens were available for examination, but because it lives in dens and is larger than the stoat, there is no reason to doubt that it, too, could be infested by all stages of the tick, provided that the distributions of the tick and pine marten coincide.

The present data reveal (i) that hedgehogs, foxes, stoats, weasels, polecats and ferrets yield females, nymphs and larvae of *I. hexagonus*; (ii) that the weasel is the smallest habitual mammalian host (confirmed by Milne's unpublished observations);

Table 4. *Human parasitism by* Ixodes hexagonus *in London and the Home Counties*

Infesting tick stage*	Date	Locality	Notes on host or locality
F.	7. x. 40	New Malden, Surrey	On back of woman
F.	4. i. 42	Wealdstone, Middx.	In house
F.	6. vii. 43	East End of London	On head of child
F.	10. vi. 42	Uxbridge, Middx.	In house
F.	26. vi. 44	Kingston, Surrey	On back of man
F.	26. vi. 44	Edgware, Middx.	On abdomen of woman
F.	27. vi. 44	Chelmsford, Essex	On a woman
N., F.	27. vi. 44	Crayford, Kent	In Anderson shelter, complaints by child of bites
F.	28. vi. 44	Esher, Surrey	On head of child
2F.	3. vii. 44	Tunbridge Wells, Kent	On head and neck of small boy
F.	7. vii. 44	Ilford, Essex	In air-raid shelter, complaints by occupants of bites
F.	10. vii. 44	Enfield, Middx.	On scapula of boy in shelter
F.	10. vii. 44	Enfield, Middx.	On forearm of man, in same shelter as boy above
F.	12. vii. 44	Caterham, Surrey	On head of child
F.	12. vii. 44	Bracknell, Berks	On head of baby
F.	19. vii. 44	Bexley Heath, Kent	In Anderson shelter, complaint by woman of bite on leg
M., F., 10N	25. vii. 44	Harrow-on-the-Hill, Middx.	In Anderson shelter, complaints by occupants of bites
L.	2. viii. 44	Wallington, Surrey	On body of child
F.	9. ix. 44	Walthamstow, London	On head of child
F.	1. vi. 45	Tottenham, London	Under breast of woman
F.	8. viii. 45	London	On man
F.	27. iv. 46	Kent	In house
N.	25. vi. 46	Stepney, London	On temple of small boy
F.	9. x. 46	Guildford, Surrey	In house
M.	20. x. 46	Dagenham, Essex	In factory
F.	28. v. 47	Dover, Kent	On man
F.	19. vi. 47	Welwyn, Herts	On chest of woman
F.	20. vi. 47	Ashtead, Surrey	On head of boy
F.	14. vii. 47	Cheltenham, Glos.	On neck of boy
F.	23. vii. 47	Letchworth	In house
F.	9. viii. 47	Mitcham, Surrey	On man
F.	9. viii. 47	Mitcham, Surrey	On girl
F.	13. iv. 50	Rochester	Scalp of female
F.	13. vii. 50	Bexley Heath	From back of patient
F. (unfed)	20. vii. 50	Sutton, Surrey	From between shoulder blades of child
F.	22. viii. 50	New Malden, Surrey	On man
N.	29. viii. 50	Whitstable, Kent	Head of child
F.	11. vii. 51	Wimbledon	Leg of woman

* F. refers to female, N. to nymph, L. to larva, M. to male.

(iii) that species bigger than the weasel, which do not live in nests or dens, are accidental hosts; and (iv) that (including the records of Milne (1949)), there is an absence of *I. hexagonus* on birds in the areas investigated.

found on the head and neck on twelve occasions, on the back three times, on the abdomen and chest twice and on the limbs twice. Female ticks were the most common but larvae and nymphs were reported occasionally. The fewer reports of larvae

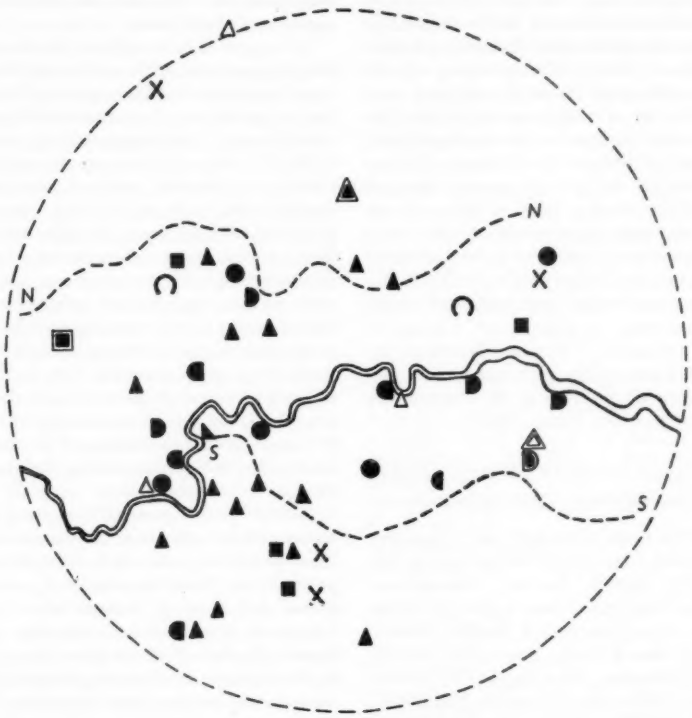

Fig. 3. Hosts of *Ixodes hexagonus* within a 20 mile radius of London. *SS*, approximate southern limit; *NN*, approximate northern limit of foxes, stoats and weasels. Duplication of records from the same host in the same locality, are shown by a line around the symbols. ■, house or building; ▲, man; ◣, stoat; ◗, dog; ●, hedgehog; ∩, air-raid shelter; △, ferret; ◖, cat; ×, host not known.

V. HUMAN PARASITIZATION BY *IXODES HEXAGONUS*

Ticks are unfamiliar as human parasites in most British districts and even when they have been found, a number of people have taken them to be warts. Sometimes, however, these ticks have been collected from human beings in London and the Home counties and then submitted to the British Museum (Natural History) for identification. The records in Table 4 have, for the most part, been obtained from this source.

Most of these ticks had commenced to feed. In seven cases we have no information on the site of attachment of the ticks on the body; ticks were and nymphs on man can probably be attributed to their shorter feeding periods and their smaller sizes, whereby they might be more easily overlooked.

Complaints of bites by individuals in air-raid shelters were reported in 1944. Whether the bites of *I. hexagonus* are painful or not is far from clear, nor have we any sound evidence that they cause inconvenience or irritation. I have fed larvae on my arm and never felt the incision, an experience shared by most people. Further the tick is not usually seen until it has ingested blood and swollen to an appreciable size. Ill effects following the bite are uncommon, although two cases of malaise have been reported elsewhere (Arthur, 1947). In one of the present records a slow healing ulcer developed

around the lesion produced by the tick. No diseased condition has been apparent in infested hedgehogs but histopathological changes in the dermis of the skin have been observed (Arthur, 1953).

Many hosts are parasitized by *I. hexagonus*, but in built-up areas dogs, cats and hedgehogs are undoubtedly the principal ones. Hedgehogs frequently wander in the suburbs of London, and occasionally these animals were found drowned in flooded Anderson shelters (Fitter, 1945). Mr Browning, of the British Museum (Natural History), suggests that the abnormal records of human parasitization by *I. hexagonus* in 1944 were probably correlated with the bombardment of Britain by VI flying bombs. More people sought refuge in garden air-raid shelters during these months than in the previous years of the war, and these shelters may have harboured the hosts of this tick at an earlier date. It is likely that cats and dogs pick up ticks in the vicinity of hedgehogs' nests and introduce them into gardens and houses. In this respect it is significant that most instances of human parasitization in the London area are closely correlated with open spaces (Fig. 3) where hedgehogs or other hosts probably do occur (see also Fitter, 1945).

VI. *IXODES HEXAGONUS* IN RELATION TO THE NESTS OF THE HOSTS

Examination of the nests of hedgehogs in January 1950 yielded the following unfed ticks: nest 1, Isle of Grain, 1 male; nest 2, Bettws, Ammanford, 1 male, 1 female, 3 nymphs; nest 3, Isle of Grain, 1 female, 1 male, 5 nymphs; nest 4, Bettws, 1 male, 4 nymphs; nest 5, Isle of Grain, 7 nymphs. Various stages of ticks (7 females, 24 nymphs, 17 larvae) were found in a compost heap at Kenton, Middlesex; a hedgehog had been hibernating in this heap which had been covered during the winter. In the five aforementioned nests the animals were also hibernating, and in no instance did we find ticks on the animal. A similar case was reported from Begbroke, near Oxford (7 November 1934) by Elton (1937), where a hedgehog was found hibernating in a pile of hay and straw. No ticks were found on the animal itself but a number were found crawling within the material. The work of Carrick & Bullough (1940) on the feeding of *I. ricinus* on hedgehogs showed that during hibernation the latter were parasitized successfully with unfed nymphs and female ticks in mid-winter as long as the temperature (10° C.) was sufficient for the ticks to be active, although owing to the lower blood temperature of the host a longer time was required to complete engorgement. Similar considerations may also apply to *I. hexagonus*.

All stages of *I. hexagonus* do occur in the nests of hedgehogs, and, with such exceptions as the large nest described by Thompson (1937), most of the records show that the number of ticks found in the nest is small. This suggests that a large proportion of the fed ticks do not drop off within the nest: the number of reported cases of so-called 'accidental' human parasitism, apart from other records, supports this contention. The following experimental data, while not conclusive, also lend support to this view. A cocker spaniel was allowed to run for some 15 min. on a fenced patch of moorland at the Isle of Grain on the dates shown in Table 5. Four hedgehogs' nests had been located on this site and marked by white markers. For 24 hr. after the run the dog was confined to the house and subsequently examined, when ticks were found on the animal. The dog gave no indication of concentrating attention on the hedgehogs' nests, but made indiscriminate runs over the patch.

Nuttall (1911) pointed out that species parasitizing wandering hosts tend to have a high fecundity, those possessing more or less fixed habitats are less productive. Thus females of *I. canisuga* produce about 400 eggs, *I. ricinus* about 1890 eggs. *I. hexagonus* lays between 880 and 1450 eggs per female (Arthur, 1951, independently confirmed by E. Browning), so that as far as fecundity is concerned it is intermediate between *I. canisuga* and *I. ricinus*.

If these ticks do drop off away from the nest, then the ability of females to produce eggs, or of the immature stages to metamorphose successfully, will depend on the microhabitat conditions in which they find themselves. Lees (1946) has shown that *I. hexagonus* (female) is more resistant to desiccation than is *I. ricinus* (female), and some of the former obviously do survive the microclimatic conditions found away from the nest and constitute a focal point for parasitizing other animals, e.g. cats, dogs

Table 5. *Tick pick-up by a cocker-spaniel at Rose Court Farm, Isle of Grain*

Date of release	Date of examination	Tick stages attached: Females	Nymphs	Larval
6 vi. 1949	7 vi. 1949	.	8	.
13 vi. 1949	14 vi. 1949	.	6	.
30 iv. 1950	1 v. 1950	2	17	29
2 vi. 1950	3 vi. 1950	.	10	13
1 viii. 1950	2 viii. 1950	.	2	.

and man (in air-raid shelters, § V). If, on the other hand, the ticks occur predominantly in the nest of the hedgehog and our data show a gross underestimation of the true position, then the mortality rate of these ticks must be very high, particularly when it is borne in mind that hedgehogs change their nests often and that in some winters, at least, the death-rate among the hibernating hedgehog stock is very high (Corley, in Elton, 1937).

The available data indicate that *I. hexagonus* is closely associated with 'nesting' mammals at and above the size-level of weasels. Apart from hedgehogs' nests, the only other record from a nest is two males and one female from a badger's sett in Windsor Forest. On the excellent evidence of Neale (1948) and of the very strong suggestion from Milne's data on *I. ricinus*, the badger is seemingly remarkably free from all kinds of ticks. It is generally accepted that *I. hexagonus* breeds in the nests of weasels, stoats and foxes, but no one appears to have produced satisfactory evidence on this point. Until such evidence is forthcoming it is conjectural to state that *I. hexagonus* finds a suitable microhabitat for survival and reproduction in such situations. A comparison of Milne's unpublished data and the present material may throw interesting light on this issue. The unpublished data for northern England showed that weasels (2 examined), stoats (5 examined), hedgehogs (5 examined) and foxes (25 adults, 31 cubs examined) were practically free of *I. hexagonus* (Table 3). The following interpretations of this data may be considered: (*a*) sampling may have been inadequate; this may possibly account for the absence of ticks on weasels, stoats and hedgehogs, but not so on foxes (56 adults and cubs examined); (*b*) sampling when this tick is not active on the host, but as seen earlier, *I. hexagonus* occurs on hedgehogs in south Wales and south-east England at the same time as *I. ricinus* does on other hosts in northern England; (*c*) *I. hexagonus* may be rare in the northern counties, as suggested in the records of Milne, although it has been found in some numbers on sheep in Ettrick (Scotland) by MacLeod (private communication) and on polecats and hedgehogs in Ross-shire by Evans, 1907 (Nuttall *et al.* 1911); (*d*) hedgehogs are the principal hosts of *I. hexagonus* in the southern counties. These hosts are apparently not common on hill and moorland grazings of northern England, and Milne (1949) collected only six specimens at five farms in 4 years. Hedgehogs prefer non-heather land with an abundance of cover and wood edges for retreat. The other major hosts, stoats, weasels, polecats and foxes, are also found there, and a plausible hypothesis would therefore be that when stalking prey they may, in their wanderings, pick up active ticks either from hedgehogs' nests or from their immediate neighbourhood. (To what extent hedgehogs make use of established tracks is not clearly known, but that might be an interesting field for inquiry.)

VII. DISTRIBUTION OF *IXODES HEXAGONUS* IN BRITAIN

I. hexagonus has been reported from a number of counties and, while the information is by no means complete, some indication of its occurrence can be seen from the list below. These data show that fewer records of *I. hexagonus* are known from the northern counties than from those in the south. This may possibly be attributed to inadequate sampling; the unpublished results of Milne (see Table 3), however, do not support this, and the differences between the north and the south appear to be valid. In the following list the figures in parentheses indicate the number of known records, which have been obtained either from literature or from personal observation:

England

Cumberland (4)
Northumberland (2)
Westmorland (2)
Lancashire (4)
Cheshire (1)
Derby (1)
Shropshire (1)
Staffordshire (1)
Warwickshire (3)
Cambridge (12)
Norfolk (1)
Suffolk (3)
Gloucester (4)
Oxford (12)
Hertford (9)
Essex (5)
Berkshire (5)
Middlesex (20)
Surrey (21)
Kent (83)
Sussex (6)
Hants (4)
Dorset (1)
Somerset (4)
Cornwall (3)
Monmouth (9)

Wales

Denbigh (1)
Merioneth (5)
Cardigan (8)
Brecon (2)
Pembroke (1)
Carmarthen (46)
Glamorgan (5)

Scotland

Ross and Cromarty (2)
Selkirk (1)
Perth (1)
Outer Hebrides (1)

VIII. SUMMARY

On the available data from south Wales and south-east England, the tick *Ixodes hexagonus* Leach has spring and autumn peaks of activity on hedgehogs, stoats and weasels.

One or more stages of this tick have been recorded on fifteen mammalian hosts. All stages have been collected from hedgehogs, stoats, foxes, polecats and ferrets. The weasel is probably the smallest habitual host. No specimens of *I. hexagonus* have been found on birds.

Man is an 'accidental' host and records of this tick on man in the London area are given. From the present information it seems that ill-effects following the bite of this parasite are rare. In built-up areas the probable sources of infestation are dogs, cats and hedgehogs. Air-raid shelters and similar constructions which have harboured these hosts at one time or another may provide a centre of infestation of man. Our observations indicate that the number of ticks in most hedgehogs' nests is not large. Nothing is known of the tick population in the habitats of other hosts. It is suggested tentatively that the ticks may drop off hedgehogs away from the nest and that after further development (i.e. hatching of eggs and metamorphoses of later stages) they are picked up by such animals as foxes, stoats, weasels.

The records of this tick in Britain are summarized and they indicate that *I. hexagonus* is a southern species.

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IXODES THEILERI N.SP., WITH OBSERVATIONS ON SPECIES CONFUSED THEREWITH

By D. R. ARTHUR, *Department of Zoology, King's College, University of London*

(With 16 Figures in the Text)

Ticks collected from birds in South Africa were sent to me for identification by Dr Gertrud Theiler of the Section of Parasitology, Department of Agriculture, Onderstepoort, South Africa. This collection consisted of partially fed or fully fed female ticks and one gorged but mutilated nymph. These specimens had previously been identified as *Ixodes brunneus* Koch or *Ixodes daveyi* Nuttall. Much confusion persists in the identification of these and closely allied species, and doubtless many of these forms have been incorrectly identified in the past. Cooley & Kohls (1945) and Arthur (1952) have indicated that in *I. brunneus* and *I. frontalis* Panzer it is necessary to remove and mount the hypostome for examination under the microscope before detailed study can be made. Examinations of the hypostome and other structures *in situ* are the probable cause of most of the inaccuracies. Therefore in the present work the hypostome, coxae and scutum were removed and examined under the microscope.

Dr Theiler informs me that the sources of this material were as follows:

2585 (i) From a migratory swallow, Mariental, south-west Africa. Three females, 1 February 1938.

2585 (ii) From *Hirudo rustica* just prior to return migratory flight to Europe, Gibeon, south-west Africa. Females, 1 February 1938.

2585 (iii) From a 'vink' (possibly a 'bishop bird' or 'weaver'). Ficksburg, Orange Free State. Females and 1 nymph, May 1942.

2585 (iv) From the neck of *Creatophora carunculata* caught by a dog, Bothaville, Orange Free State. Females, October 1951.

2444 (i) From *Quelea sanguinirostris lathami*, Onderstepoort. One female, November 1919.

All these specimens belong to one species, to which I propose giving the name of *I. theileri* n.sp. for reasons given later. The present description is based on the above material.

DESCRIPTION OF FEMALE

Body. Oval, wider posterior to the middle. Well-fed females may measure 10 by 7 mm. and are wider behind the spiracle. Colour of well sclerotized parts dark brown in preserved specimens. Post-scutal and ventral regions with numerous short, curved (in preserved material at least) white hairs.

Capitulum (Figs. 1 and 2). Length 0·87 mm., breadth 0·60 mm. Lateral profile lines slightly undulate. Posterior margin a little concave, salient. Cornua short but evident as distinct postero-lateral projections. Porose areas large, extending from dorsal ridge almost to the base of the palps, and thence to the lateral borders; elongate oval, superficial. Palps (Fig. 4) long, usually rounded distally but in some preparations they appear to be pointed. Length of article II to article III as 2·3 : 1·8. Combined lengths of article II and III about 0·75 mm. Outer profile lines straight, although in some specimens gently concave; inner edges mildly convex. Outer surface of article II irregular, with a pronounced indentation basally. Article II broader than article III which tapers from its base. Article I broader than long, with a mesal-ventral plate-like projection arising from palpal article I. Article II flat, article III slightly hollowed out mesially, with a ventro-mesal knob-like projection below article IV. This projection occurs also in *I. brunneus* and *I. frontalis*.

Ventrally, the basis is broad and roughly rectangular in shape; constricted behind the auriculae, which are prominent lateral extensions terminating in sharp straight edges with two corners. Posterior margin straight or very gently curved, salient.

Hypostome (Fig. 3). Length from basal tooth to tip varies from 0·50 to 0·59 mm. (mean 0·54 mm.). Narrow and pointed. Denticles arranged from distal to proximal end in about 14 rows of 4/4; 3 rows of 3/3 (or 2 rows of 3/3); 1 row of 2/2 (or 2 rows of 2/2). Ventral median area unarmed.

Scutum (Fig. 6). Scutum with pointed scapulae, moderately emarginate. Mean length 1·58 by 1·17 mm., maximum and minimum ranges 1·71 × 1·27 to 1·49 × 1·09 mm. Rounded lateral angles; widest in front of the middle, converging behind, posterior margin rounded. Cervical grooves as broad valleys separating the lateral and median areas; converging slightly in front and diverging behind, fading out before reaching the postero-lateral margins. Lateral carinae short and steep. Surface finely punctate; rugosities in cervical groove and in the lateral area. Bristles between scapulae

short, fine and pointed; bristles in lateral field and in the mid-region of the median area, more or less parallel-sided and serrate distally. Preponderance of dermal duct apertures in the more heavily pigmented lateral and postero-median fields.

Genital aperture between coxae III.

Anal groove, in partially fed females, is horse-shoe shaped, but in well-gorged specimens is drawn out longitudinally. Groove ceases in front of the posterior margin of the body.

Spiracle (Fig. 5). Short, oval with longer axis slightly oblique to the transverse plane. Surface nearly level, elevated. Macula eccentric, antero-ventral. Length 0·41 mm., breadth 0·34 mm.

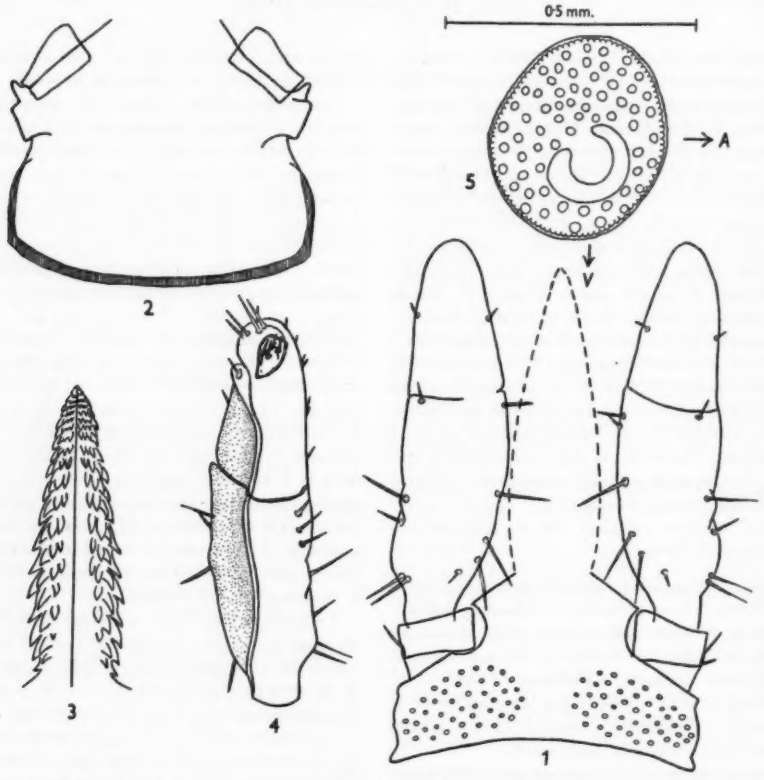
Legs (Figs. 7 and 8). Long and large, supplied with long hairs. Tarsus I (Fig. 7*a*) broad and long, with a slight hump in front of the anterior trough of Haller's organ, after which it tapers more rapidly to the apex than it does proximally. Length of tarsus I, 0·86 mm.; metatarsus I, 0·52 mm.; tarsus IV, 0·75 mm.; metatarsus IV, 0·60 mm. Bristles numerous, long and conspicuous; tarsal bristles ending in single point, most of the bristles on remaining segments parallel sided with distal serrations.

Coxae (Fig. 8). Mildly convex, shining; bristles few and long, serrate distally. Coxa I and II close together, the others further apart. Internal spur on coxa I longer and more tapered than the external spur. Internal spurs on coxae II–IV present as rounded thickenings of the coxal margin. External spurs on all coxae prominent, usually with rounded extremities.

Trochantal spurs present, short, usually decreasing in size from legs I to III. Absent from trochanter IV.

Processus coxalis and processus cymati well developed.

The species is named after Dr G. Theiler who submitted the specimens to me, and the type collection (2585 iv) is now preserved in the Onderstepoort Research Station, South Africa.



Figs. 1–5. *I. theileri*. Fig. 1. Female. Capitulum, dorsal view. Fig. 2. Female. Basis, ventral view. Fig. 3. Female. Hypostome. Fig. 4. Female. Palp. Fig. 5. Female. Spiracle.

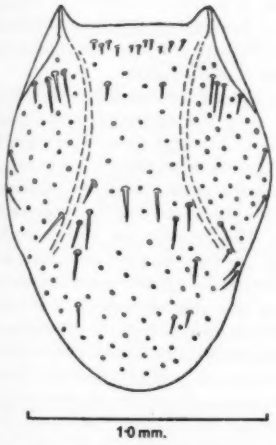
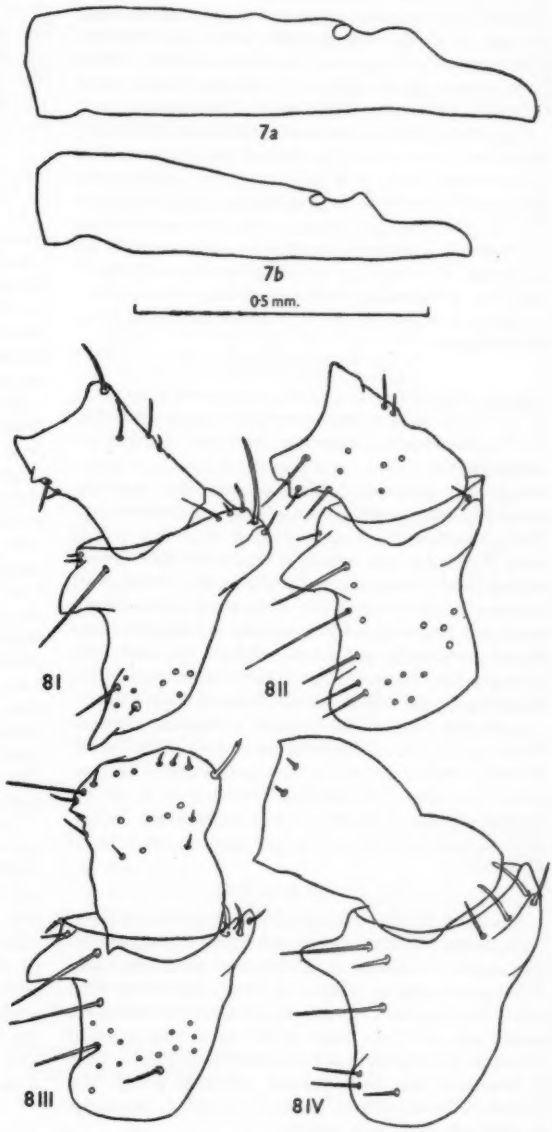


Fig. 6. *I. theileri*. Female. Scutum.



Figs. 7, 8. *I. theileri*. Fig. 7. (*a*) Female. Tarsus. (*b*) *I. frontalis*. (in *sensu* Arthur, 1952) female. Tarsus. Fig. 8. *I. theileri* female. Coxae (I), (II), (III), (IV).

DISCUSSION

A number of species of the genus *Ixodes* have been assigned to the same species on their gross morphology without due consideration for detailed structure. Four species, *I. daveyi* Nuttall 1913, *I. brunneus* Koch 1844, *I. frontalis* Panzer and *I. thelieri*, have been confused in this manner.

The South African material [collection 2585 (ii)] described in the present work had been identified as *I. brunneus* Koch 1844 by R. du Toit, which was confirmed by workers at Cambridge, and collections 2585 (i), (iii) and (iv) hence were also identified as *I. brunneus*. Bedford identified collection 2444 as *I. daveyi*. To clear up existing doubts and inaccuracies, additional notes on certain species, viz. *I. daveyi*, *I. brunneus* and *I. frontalis*, are thought to be necessary.

(*a*) Ixodes daveyi

Most of the original figures given by Nuttall (1913) are accurate representations of the type specimen in the British Museum (Natural History). Having re-examined this, however, I find the following amendments are necessary. The cornua are distinct, rounded, backwardly projecting protuberances. The hypostome is long, tapering to a fine point, with 21–22 distinct teeth on the outer files. These hypostomal teeth are arranged from base to apex in 2 rows of 3/3 and then 4/4 for the entire length. These teeth are separated by a median triangular, unarmed surface for the proximal half. The external spurs on the coxae are not 'sharp' as described by Nuttall (*loc. cit*) but resemble those of *I. frontalis*.

A female tick in the Nuttall collection (British Museum, Cat. no. 150) found on a dove (*Haplopelia johnstoni* Shelly) from the Mlange mountain, Nyasaland, was identified as *Ixodes brunneus* Koch by Nuttall and as *I. frontalis* by Pomerantzev (1950). A close examination of the specimen shows it to be *I. daveyi*.

(*b*) Ixodes brunneus

Cooley & Kohls (1945) have expressed the opinion that in general tick species are limited geographically to the continent on which they were originally indigenous, unless there is a land connexion with other continents. They admit, however, that natural migration of birds may serve as a transporting medium for ticks, but think it improbable that *I. brunneus* has been carried on birds across the Atlantic Ocean. These authors accept *I. brunneus* as a North American species.

Boero (1945) described a species under the name of *I. brunneus* from the Argentine, but Ringuelet (1947) has shown this to be incorrect and has redescribed Boero's species under the name of *I. neuquenensis*.

For comparison with *I. thelieri*, Dr Glen M. Kohls loaned me the following specimens of *I. brunneus*:

(i) '♀ ex *Larius l. anthonyi*, Santa Catalina Island, Los Angeles Co. California, 29 Feb. 1940 (17801).'

(ii) '♀ ex Myrtle Warbler, Phillipy, Lake Co. Tennessee, 11 April 1941 (19346).'

(iii) '♀ ex Slate coloured junco, North Little Rock, Arkansas, 1 Feb. 1944 (20541).'

(iv) '♀ ex White throated sparrow, North Little Rock, Arkansas, 17 April 1944 (20936).'

(v) '♀ ex Slate coloured junco, North Little Rock, Arkansas, 24 April 1944 (20938).'

In reviewing the *brunneus* material Kohls (private communication, 21 November 1952) points out that the median ventral unarmed area of the hypostome extends further forwards than depicted in their monograph (fig. 79 C, Cooley & Kohls, 1945), and that there appears to be some variation in this respect. The hypostome is somewhat more sharply pointed than in their figure.

Mounts made of coxae and trochanters of specimen 20936 above show that the external coxal spurs (legs I–IV) and the trochanter spurs (legs I–III) (Figs. 13*a–d*) are larger than originally indicated, a view to which Kohls subscribes. Both the processus coxalis and processus cymati are well developed and bear stout bristles. The bristles on the posterior margins of the coxae are very slender and many are inordinately long; similar long bristles are evident at the distal margin of the trochanters. The length-breadth ratio of the scutum is as 1·43 : 1·0 (measured after removal from the tick). The lateral field is more heavily pigmented than the postero-median central area, and the latter more so than the antero-median portion. Lateral carinae are absent. The sides curve gently to a broad posterior convex margin (Fig. 14). The bristles are large, long and conspicuous, and appear to terminate distally in two or more points.

Ventrally the basis capituli is broadly rounded, and this is accentuated by lateral chitinous thickenings as depicted in Fig. 11. The auriculae in the specimens at my disposal terminate laterally with straight edges and two corners. Basally the sides of the auriculae converge somewhat, thus producing a wedge-shaped structure. Distally and on the upper surface of the auricula there is a V-shaped depression (Fig. 12*a*). Behind the auriculae the basis is distinctly constricted. As in *I. fronialis* and *I. theileri*, a ventral plate-like extension arises from the basal palpal article.

(*c*) Ixodes frontalis

Recently, Pomerantzev (1950) described a species from Russia under the name of *I. frontalis* (Panzer) and gave its distribution as Western Europe, France, Germany, Italy and Africa. He relegated the North American *brunneus* to the subspecific rank of *I. frontalis brunneus* and in this he has followed

Schulze (1933). The anal grooves of *I. frontalis* (*sensu* Pomerantzev) are horseshoe-shaped in unfed females, but in both *I. frontalis* (*sensu* Arthur, 1952) and *I. brunneus* (*sensu* Cooley & Kohls, 1945) the anal grooves are parallel-sided: this is in agreement with the description given by Neumann (1899) and by Nuttall, Warburton, Cooper & Robinson (1911).

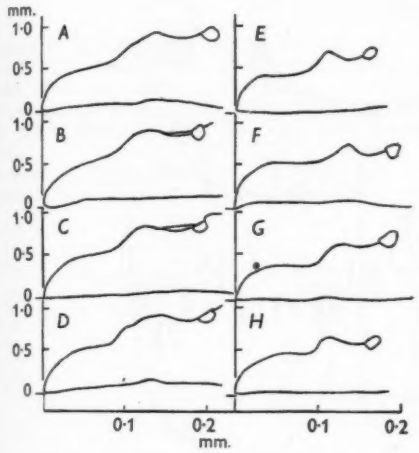


Fig. 9. *I. theileri* female: tip of tarsus I (*A–D*). *I. frontalis* (in *sensu* Arthur, 1952) female: tip of tarsus I (*E–H*). These represent the major variations in the two species.

Moreover, the latter workers (Nuttall *et al.*) wrote 'the anal grooves parallel or slightly divergent'. The investigations of Cooley & Kohls leave no doubt that Nuttall *et al.* confused *I. brunneus* and *I. frontalis*. Is Pomerantzev, in the same way, describing *I. frontalis* and *I. theileri* (or a similar species) as one species? In many features *I. frontalis* (in *sensu* Pomerantzev) resembles *I. theileri* and, if not synonymous, is obviously closely related to it. Further comment is superfluous until such time as the Russian collection can be examined.

Allowing for intra-specific variation, size may be a possible subsidiary factor in the separation of these four species. In this respect the data in Table 1 and Fig. 9 are of some interest. The dimensions of the hypostome, tarsus I and the scutum (i.e. the more easily measurable structures) indicate that *I. theileri* is larger than either *I. frontalis* or *I. brunneus*; the differences being particularly evident in the scutum and the first tarsal joint.

Separation of these four species can be made by reference to the following key:

1. Anal groove horshoe-shaped. 2.
   Anal groove parallel-sided. 3.
2. Scutum with posterior margin rounded. Bristles absent. Genital aperture between coxae IV. *I. daveyi*.
   Scutum with lateral margins converging posteriorly. Bristles present. Genital aperture between coxae III. *I. theileri*.
3. Scutum suboval, lateral margins gently curving behind the middle. Posterior margin broadly rounded. *I. brunneus*.
   Scutum with rounded lateral angles. Lateral margins converging posteriorly. *I. frontalis*.

Differences between certain characters are, however, better indicated by a comparison of the figures. Thus in *I. theileri* the hump immediately in front of the anterior trough of Haller's organ is longer and has a gentler slope than in the tarsi of *I. frontalis*. The region in front of the hump in *I. theileri* shows a gradual slope to its tip, whereas in *I. frontalis* the corresponding region is flatter, before tapering abruptly to the pulvillus (Figs. 7*a*, *b*; 9). Similarly, the form and size of the spurs on the coxae and trochanters are more clearly indicated by comparing Fig. 13 (*a–d*), Fig. 15 (*a–d*) and Fig. 8 (I–IV).

SUMMARY

1. A new species of tick, *I. theileri* from South Africa, is described. Hitherto it has been collected only from birds.

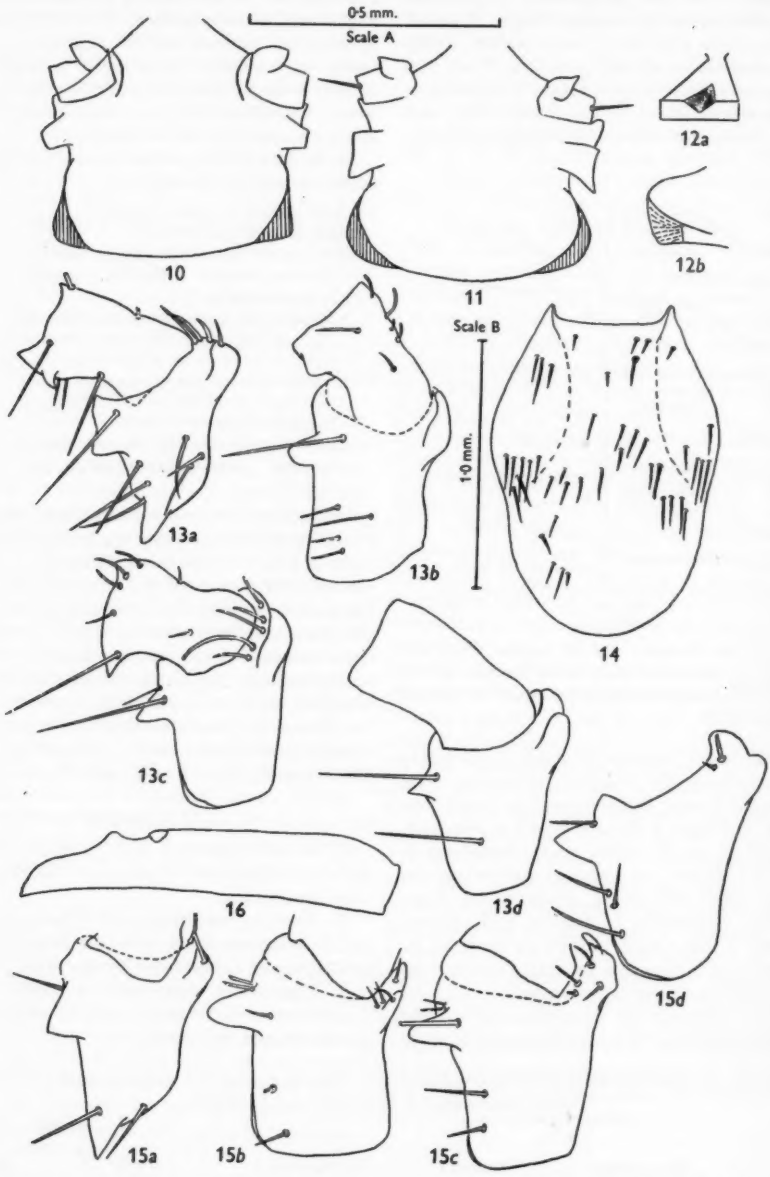
2. Existing descriptions of *I. daveyi* Nuttall 1913 and *I. brunneus* Koch 1844 are amplified, and the synonymy of *I. frontalis* (Panzer, 1795) discussed.

3. Diagnostic characters, whereby *I. daveyi*, *I. brunneus*, *I. frontalis* and *I. theileri* may be separated, are tabulated.

Table 1. *Measurements of the scutum, hypostome and the first tarsal and metatarsal joints of* Ixodes brunneus, I. frontalis *and* I. theileri

		Hypostome			Tarsus I			Metatarsus I			Scutum: Length			Scutum: Breadth		
Species		S*	M†	L‡	S	M	L	S	M	L	S	M	L	S	M	L
I. brunneus		—	0·432	—	—	0·75	—	—	0·39	—	1·30	—	1·50	0·96	—	1·05§
I. frontalis		0·47	0·49	0·50	0·75	0·79	0·81	0·44	0·46	0·48	1·42	1·47	1·49	1·02	1·05	1·07
I. theileri		0·50	0·54	0·59	0·81	0·86	0·89	0·49	0·52	0·53	1·49	1·58	1·71	1·09	1·17	1·27

S* = shortest length. M† = mean length. L‡ = longest length.
§ After Cooley & Kohls (1945).



Figs. 10–16. Fig. 10. *I. frontalis*. Basis capituli, ventral view. (Drawn to scale A.) Fig. 11. *I. brunneus*. Basis capituli, ventral view. (Drawn to scale A.) Fig. 12. (*a*) *I. brunneus*. Schematic representation of the auricula from the upper surface. (*b*) *I. frontalis*. Schematic representation of the auricula from the upper surface. Fig. 13. (*a–d*). *I. brunneus*. Coxae and trochanters of legs I–IV. (Drawn to scale A.) Fig. 14. *I. brunneus*. Scutum after removal and mounting on a slide. (Drawn to scale B.) Fig. 15. (*a–d*). *I. frontalis*. Coxae of legs I–IV. (Drawn to scale A.) Fig. 16. *I. brunneus*. Tarsus of leg I. (Drawn to scale A.)

Grateful acknowledgement is made to Dr Gertrud Theiler, Parasitologist, Department of Agriculture, Onderstepoort, South Africa, for the opportunity to examine these ticks. Dr Theiler was also generous enough to check my descriptions against the specimens, and I thank her for her invaluable comments. Dr Glen M. Kohls very kindly read the original draft and loaned me material of *I. brunneus* for checking purposes. With their customary kindness Dr G. Owen Evans and Mr E. Browning gave me every facility for examining the tick collections in the British Museum, and I am deeply indebted to them. In preparing the paper for publication, Dr P. Tate kindly advised me on a number of points.

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THE CHEMOTHERAPY OF *PLASMODIUM BERGHEI*. I. RESISTANCE TO DRUGS

By JUNE P. THURSTON*, *The National Institute for Medical Research, London, and the Molteno Institute, University of Cambridge*

When a strain of *Plasmodium gallinaceum* had been made resistant to proguanil, Bishop & McConnachie (1948) found that it was also resistant to sulphadiazine. This discovery was followed by reports of many other investigations of cross-resistance in several species of *Plasmodium*. The results are summarized in Table 1. Cross-resistance has been reported between sulphonamides, proguanil (Paludrine), bromoguanide, pyrimethamine (Daraprim) and some 2 : 4-diaminopteridines, but not between any of these drugs and mepacrine, quinine or the quinoline antimalarials. There are, however, several conflicting reports which emphasize the differences between the species of *Plasmodium* and also between different resistant strains of a single species.

This paper describes the production of strains of *P. berghei* resistant to sulphadiazine, pyrimethamine and methylene blue, and an investigation of the sensitivity of these resistant strains to other antimalarial drugs. These include M3349, 2-*p*-chlorophenyl-guanidino-4-β-diethylamino-ethylamino-6-methylpyrimidine, which was one of the precursors of proguanil; CTW6, 2 : 4-diamino-5-*p*-chlorophenylpyrimidine, which differs from pyrimethamine only in the absence of the ethyl group on the 6- position of the pyrimidine ring (Chase, Thurston & Walker, 1951); CPT, 2 : 4-diamino-1-*p*-chlorophenyl-1 : 6-dihydro-6 : 6-dimethyl-1 : 3 : 5-triazine, which is an active metabolite of proguanil (Carrington, Crowther, Davey, Levi & Rose, 1951; Crowther & Levi, 1953); and four of a series of 2 : 4-diaminopteridines which were tested by Collier & Waterhouse (1950, 1952) for vibriostatic and antibacterial activity—these are 0/97 (6 : 7-camphano-), 0/103 (6 : 7-di*n*hexyl-), 0/129 (6 : 7-di*iso*propyl-) and 0/164 (6 : 7-(1′-ethylindolo)-2 : 4-diaminopteridine).

* Tucker-Price Research Fellow of Girton College. Present address: The Molteno Institute, Downing Street, Cambridge.

Table 1. *The sensitivity of resistant strains of malaria to the action of other antimalarials*

Drug to which resistance was induced	Species of malaria parasite	Drugs to which the strain was also resistant	Drugs to which the resistant strain was still sensitive	References
Proguanil	*P. gallinaceum*	M4430	Quinine, mepacrine, pamaquin, sulphadiazine and M3349	Williamson & Lourie (1947); Williamson, Bertram & Lourie (1947)
	P. gallinaceum	M4430	Mepacrine and pamaquin	Bishop & Birkett (1947, 1948)
	P. gallinaceum	Sulphadiazine	—	Bishop & McConnachie (1948)
	P. gallinaceum	—	Metachloridine	Bishop & McConnachie (1953)
	P. gallinaceum	—	Hypersensitive to sulphadiazine	Greenberg (1949)
	P. gallinaceum	2 : 4-Diamino-6 : 7-diphenylpteridine	2 : 4-Diamino-5(*p*-chlorophenoxy)-6-methylpyrimidine	Greenberg & Richeson (1951)
	P. gallinaceum	2 : 4-Diamino-6 : 7-diphenylpteridine	Slightly hypersensitive to pteridine 0/129	McConnachie (1953)
	P. gallinaceum	—	Pyrimethamine	Rollo (1951)
	P. gallinaceum	Pyrimethamine	—	Robertson, Davey & Fairley (1952)
	P. lophurae	—	Quinine, mepacrine, chloroquin, camoquin and pamaquin	Thompson (1948)
	P. berghei	—	Sulphadiazine, pyrimethamine	Rollo (1951)

Table 1 (*cont.*)

Drug to which resistance was induced	Species of malaria parasite	Drugs to which the strain was also resistant	Drugs to which the resistant strain was still sensitive	References
Proguanil	*P. knowlesi*	Pyrimethamine, bromoguanide	Sulphadiazine, M 3349 and chloroquin	Singh, Ray, Basu & Nair (1952)
	P. cynomolgi	—	Mepacrine, chloroquin and pamaquin	Schmidt, Genther, Fradkin & Squires (1949)
	P. cynomolgi	—	Sulphadiazine and pyrimethamine	Hawking & Thurston (1951 and unpublished result)
	P. vivax	—	Chloroquin and sulphadiazine	Cooper, Coatney & Imboden (1950)
	P. falciparum	Pyrimethamine	—	Robertson, Davey & Fairley (1952)
	P. falciparum	—	Pyrimethamine	Wilson & Edeson (1953)
Sulphanilamide	*P. gallinaceum*	Proguanil, sulphathiazole, sulphapyridine and sulphadiazine	—	Bishop & McConnachie (1950*b*)
	P. gallinaceum	—	Metachloridine	Bishop & McConnachie (1953)
Sulphadiazine	*P. gallinaceum*	Proguanil, M 4430, sulphathiazole, sulphapyridine and sulphanilamide	Quinine and mepacrine. Hypersensitive to pamaquin	Bishop & McConnachie (1950*a*)
	P. gallinaceum	—	Slightly hypersensitive to pteridine 0/129	McConnachie (1953)
	P. gallinaceum	—	Metachloridine	Bishop & McConnachie (1953)
	P. berghei	Proguanil	Pyrimethamine	Rollo (1951)
	P. berghei	Sulphanilamide, proguanil, CPT, pyrimethamine, and pteridines 0/120 and 0/103	Quinine, mepacrine, chloroquin, pamaquin, methylene blue and M 3349	Thurston (this paper)
Pyrimethamine	*P. gallinaceum*	Proguanil	—	Rollo (personal communication)
	P. berghei	Proguanil	—	Rollo (personal communication)
	P. berghei	Proguanil and CPT, CTW 6, and pteridines 0/97 and 0/164	—	Thurston (this paper)
	P. cynomolgi	Proguanil	Chloroquin	Schmidt & Genther (1953)
	P. vivax	Proguanil	—	Coatney (quoted by Schmidt & Genther, 1953)
Pteridine 0/129	*P. gallinaceum*	Proguanil	—	Bishop (personal communication)
Metachloridine	*P. gallinaceum*	Sulphadiazine, sulphapyridine, and sulphathiazole	Proguanil sulphanilamide, pamaquin, mepacrine, quinine and chloroquin	Bishop & McConnachie (1953)

METHOD OF INDUCING RESISTANCE IN *PLASMODIUM BERGHEI*

Two methods of inducing resistance to sulphadiazine were followed. In the first, mice inoculated with 10 million parasites were then treated once daily for 4 days with a dose of sulphadiazine which was slightly lower than the minimum effective dose. Fresh mice were then subinoculated with blood from the mouse with the heaviest infection. The dose of drug was increased as the strain became resistant.

In the second method, infected mice were treated daily with sulphadiazine when more than 5 % of the erythrocytes in the peripheral blood were parasitized. Blood films were examined on alternate days and the dose of drug for each mouse was increased or decreased according to the height of the infection. Fresh mice were subinoculated from the mice in which the infection had been unaffected by large doses of sulphadiazine.

The strain which was treated by the second method became resistant more quickly than the first strain, which was discontinued. Using the second method, other strains of *P. berghei* were treated continuously with pyrimethamine and methylene blue, and became resistant to these drugs. Rollo also found that a strain of *P. berghei* became resistant to pyrimethamine very slowly when the mice were treated soon after inoculation (Rollo, 1951), but that resistance appeared rapidly when mice with a patent infection were treated with the drug (Rollo, 1952*a*, *b*).

When strains were tested for their degree of resistance, or for their sensitivity to other drugs, mice were inoculated with 1 million parasites of either the resistant or the parent strain of *P. berghei*. Both strains were then treated for 4 days with doses of drug which were approximately the minimum effective dose (i.e. the lowest dose which reduced the parasitaemia of the treated mice to one-fiftieth of that of the controls) and the degree of activity was compared. The rate of formation of resistant strains is shown in Table 2. The strain treated with sulphadiazine showed 100-fold resistance after 12 months, the strain treated with pyrimethamine showed ten-fold resistance after 5 months, and the strain treated with methylene blue was slightly resistant after 4 months. The strain resistant to sulphadiazine has now retained this resistance for a further 10 months without any further treatment.

ANTAGONISM OF SULPHADIAZINE IN THE SULPHADIAZINE-RESISTANT STRAIN OF *PLASMODIUM BERGHEI*

It was found that the same doses of *p*-aminobenzoic acid and folic acid antagonized the action of the minimum effective dose of sulphadiazine against the resistant strain as against the parent strain, even though this dose of sulphadiazine was 30 times greater in the resistant strain than in the untreated strain. That is, in the parent strain sulphadiazine 0·01 mg./20 g., given intraperitoneally once daily, was antagonized by *p*-aminobenzoic acid 0·3 mg./20 g., given orally, twice daily, or folic acid 4·0 mg./20 g., orally, twice daily. In the sulphadiazine-resistant strain, sulphadiazine 0·3 mg./20 g. was similarly antagonized by 0·3 mg. *p*-aminobenzoic acid or 4·0 mg. folic acid.

Ivánovics (1942) described a similar relationship in bacteria *in vitro*. One molecule of *p*-aminobenzoic acid normally antagonized 14 molecules of sulphadiazine, but would antagonize 220 molecules in a resistant strain. Strains of *Staphylococcus* resistant to sulphonamides were found to produce up to 100 times more *p*-aminobenzoic acid than normal strains (Landy, Larkum, Oswald & Streightoff, 1943; Spink, Wright, Vivino & Skeggs, 1944), and it has been suggested that this increased production of *p*-aminobenzoic acid is the mechanism whereby bacteria become resistant to sulphonamides. The mechanism of resistance in malaria parasites is as yet unknown, but may be similar to this, with increasing amounts of drug being counteracted by increased production of natural metabolite. The

Table 2. *Degree of resistance in* Plasmodium berghei *at various times after treatment was commenced*

Strain of *P. berghei*	Length of time of treatment	Drug tested	Minimum effective dose (mg./20 g.)	Degree of resistance
Parent	—	Sulphadiazine	0·005	—
Sulphadiazine-treated	4 months	Sulphadiazine	0·025	×5
	7 months	Sulphadiazine	0·1	×20
	12 months	Sulphadiazine	0·5	×100
Parent	—	Pyrimethamine	0·01	—
Pyrimethamine-treated	2 months	Pyrimethamine	0·05	×5
	5 months	Pyrimethamine	0·1	×10
Parent	—	Methylene blue	0·3	—
Methylene blue-treated	2 months	Methylene blue	0·5	×1·66
	4 months	Methylene blue	0·7	×2·33

relation between active doses of sulphadiazine and the amount of *p*-aminobenzoic acid necessary for inhibition would then remain the same, whether the drug had a high or low threshold of activity.

SENSITIVITY OF RESISTANT STRAINS OF *PLASMODIUM BERGHEI* TO OTHER DRUGS

Sulphadiazine-resistant strain

This strain was as sensitive as the parent strain to quinine, mepacrine, chloroquin, pamaquin, methylene blue and M3349. It was cross resistant to sulphanilamide, proguanil and CPT, pyrimethamine and the pteridines 0/103 and 0/129 (Table 3). Although this strain showed 100-fold resistance to sulphadiazine, there was only ten-fold resistance to sulphanilamide, and increasing the dose of CPT from 0·01 to 0·02 mg. nearly obliterated all signs of cross-resistance.

Pyrimethamine-resistant strain

This strain was cross-resistant to proguanil and CPT, pteridines 0/97 and 0/164, and also CTW 6 (Table 4). Resistance to CTW 6 was slightly more than six-fold, and to 0/164 it was more than four-fold, although to CPT it was little more than two-fold.

Methylene blue-resistant strain

This strain was as sensitive as the parent strain to the action of pamaquin, mepacrine, sulphadiazine, proguanil, pyrimethamine and pteridine 0/97.

DISCUSSION

Plasmodium berghei rapidly became very resistant to sulphadiazine and pyrimethamine when exposed continuously to low doses of these drugs; in this it resembled other malaria parasites. Resistance to methylene blue was less marked, and this strain retained its normal sensitivity to the other anti-

Table 3. *Cross-resistance in sulphadiazine-treated strain of* Plasmodium berghei

		First experiment		Second experiment	
Strain	Drug tested	Dose (mg./20 g.)	Parasitaemia*	Dose (mg./20 g.)	Parasitaemia*
Treated	Sulphanilamide	3·0	5	—	—
	—	—	83	—	—
Parent	Sulphanilamide	0·3	8	—	—
	—	—	105	—	—
Treated	Proguanil	0·2	133	0·15	34
	—	—	366	—	200
Parent	Proguanil	0·2	10	0·15	0
	—	—	310	—	192
Treated	CPT	0·01	141	0·02	5
	—	—	675	—	200
Parent	CPT	0·01	0	0·02	0
	—	—	630	—	192
Treated	Pyrimethamine	0·005	21	0·01	35
	—	—	85	—	90
Parent	Pyrimethamine	0·005	0	0·01	0
	—	—	80	—	74
Treated	0/129	0·8	45	—	—
	—	—	85	—	—
Parent	0/129	0·8	0	—	—
	—	—	80	—	—
Treated	0/103	0·8	115	—	—
	—	—	90	—	—
Parent	0/103	0·8	0	—	—
	—	—	74	—	—

* Expressed as the average number of parasitized erythrocytes per 1000 erythrocytes in groups of three to five mice examined 5–7 days after inoculation.

The dose of proguanil was given twice daily, the other drugs once daily.

Please see introduction for formulae of CPT, 0/129, and 0/103.

Table 4. *Cross-resistance in pyrimethamine-treated strain of* Plasmodium berghei

		First experiment		Second experiment	
Strain	Drug tested	Dose (mg./20 g.)	Parasitaemia*	Dose (mg./20 g.)	Parasitaemia*
Treated	Proguanil	0·2	46	0·2	20
	—	—	92	—	30
Parent	Proguanil	0·2	4·6	0·2	1
	—	—	85	—	26
Treated	CPT	0·005	13	0·01	7
	—	—	516	—	516
Parent	CPT	0·005	1	0·01	0
	—	—	527	—	527
Treated	CTW 6	0·1	70	0·2	13
	—	—	85	—	70
Parent	CTW 6	0·05	4	0·1	0
	—	—	67	—	83
Treated	0/97	1·0	20	1·0	23
	—	—	85	—	196
Parent	0/97	1·0	1	1·0	2
	—	—	92	—	210
Treated	0/164	0·8	71	3·0	10
	—	—	172	—	90
Parent	0/164	0·8	0	1·0	0
	—	—	180	—	72

* Expressed as the average number of parasitized erythrocytes per 1000 erythrocytes in groups of three to five mice examined 5–7 days after inoculation.

The dose of proguanil was given twice daily, the other drugs once daily.

Please see introduction for formulae of CPT, CTW 6, 0/97, and 0/164.

malarials that were tested. The strains of *P. berghei* resistant to sulphadiazine and pyrimethamine were cross-resistant to all the other antimalarial drugs which have been shown (Thurston, 1953) to act upon the dividing nucleus of the parasite. There was no cross-resistance between the sulphadiazine-resistant strain and any of the quinoline antimalarials.

McConnachie (1953) found that sulphadiazine- and proguanil-resistant strains of *P. gallinaceum* were also resistant to 2 : 4-diamino-6 : 7-diphenylpteridine but were hypersensitive to 0/129, di*iso*propylpteridine. In contrast, the present sulphadiazine-resistant strain of *P. berghei* was cross-resistant both to 0/129 and to 0/103, and the pyrimethamine-resistant strain was resistant to 0/97 and 0/164; these pteridines all seem to act in a similar way against *P. berghei*.

In Tables 3 and 4 it is shown that low doses of drug that control the infection in the parent strain of *P. berghei* are ineffective against the resistant strains. No attempt was made to determine the minimum effective dose of all the drugs against the resistant strains, but cross-resistance to the proguanil metabolite (CTP) was slight in both strains, and resistance to sulphanilamide was only ten-fold in the strain showing 100-fold resistance to sulphadiazine. Resistance to the drug with which the strain was treated was in these cases much greater than the cross-resistance to the other drugs. When Rollo (1951, 1952*a*) obtained two- to three-fold resistance to pyrimethamine in proguanil-resistant *P. gallinaceum* and three-fold resistance in *P. berghei* he commented that the drug was almost as active against the resistant as against the normal strains. These figures should also be a further indication that cross-resistance does occur between these drugs.

During the early stages of inducing resistance to sulphadiazine in *P. berghei*, the strain was tested at intervals for resistance to proguanil. There was no indication that this appeared before the sulphadiazine resistance, as was described by Bishop & McConnachie (1950*b*) in *P. gallinaceum*.

The present experiments suggest that the sulphonamides, pyrimethamine, proguanil and the 2 : 4-diaminopteridines act against *P. berghei* in similar ways, because cross-resistance occurs between them all.

SUMMARY

1. Strains of *P. berghei* resistant to sulphadiazine, pyrimethamine, and methylene blue were produced

by treating acute infections with low doses of drug.

2. The strain resistant to methylene blue was sensitive to pamaquin, mepacrine, sulphadiazine, proguanil, pyrimethamine, and 2 : 4-diamino-6 : 7-camphano-pteridine.

3. The pyrimethamine-resistant strain was cross-resistant to proguanil and its active metabolite CPT, 2 : 4-diamino-6 : 7-camphanopteridine, 2 : 4-diamino-6 : 7-(1′-ethylindolo)-pteridine, and 2 : 4-diamino-5-*p*-chlorophenylpyrimidine.

4. The sulphadiazine-resistant strain was cross-resistant to pyrimethamine, sulphanilamide, proguanil and its active metabolite CPT, 2 : 4-diamino-6 : 7-di*n*hexylpteridine, and 2 : 4-diamino-6 : 7-di-*iso*propylpteridine. It was as sensitive as the parent strain to quinine, mepacrine, chloroquin, pamaquin, methylene blue, and M 3349.

5. The action of sulphadiazine against the sulphadiazine-resistant strain was inhibited by the same doses of *p*-aminobenzoic acid and folic acid as were required with the parent strain, although the dose of sulphadiazine was increased 30-fold.

I am indebted to Dr L. G. Goodwin and Burroughs Wellcome Ltd. for supplies of pyrimethamine; to Dr D. G. Davey and Imperial Chemical (Pharmaceuticals) Ltd., for proguanil and its active metabolite CPT; and to Dr H. O. J. Collier and Messrs Allen and Hanburys for the series of 2 : 4-diaminopteridines.

I also wish to thank Dr F. Hawking for his advice in this work, and Miss M. Cameron for technical assistance. Part of this work was contained in a thesis submitted to the University of London in April 1952.

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STUDIES ON THE MORPHOLOGY AND BIOLOGY OF *ENTAMOEBA MOSHKOVSKII* TSHALAIA, 1941

By R. A. NEAL, Ph.D.

Wellcome Laboratories of Tropical Medicine, London

(With 43 Figures in the Text)

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I. INTRODUCTION

Entamoeba moshkovskii was first discovered and named as a distinct species by Tshalaia working in Moscow, in 1941. As this amoeba is apparently free-living, it is of great interest to investigate its morphology and ecology in order to determine its taxonomic relationship with other species of *Entamoeba* and, in addition to the academic problems involved, it is of practical importance in connexion with the epidemiology of *E. histolytica*. It was therefore decided to try and isolate *E. moshkovskii* in this country, and these attempts were successful with the first samples of sewage that were collected.

In her first publication, Tshalaia (1941) described the isolation of *E. moshkovskii* from sewage collected at various points of the sewage disposal plant in Moscow. The amoeba was grown *in vitro* in various media suitable for intestinal Protozoa, in which it grew easily at temperatures of 10–15° C. and 37° C., and encysted in large numbers. Morphologically, *E. moshkovskii* closely resembled *E. histolytica* throughout the entire life-cycle including excystment and metacystic development. The amoebae varied in size from 10 to 120 μ, with an average of 25 μ, while cysts varied from 5 to 16 μ with an average of 10 μ. If the amoebae were placed in hypotonic solutions such as hay infusions or tap water, Tshalaia observed vacuoles which she claimed functioned as contractile vacuoles. The amoebae and cysts were not infective to kittens.

Six years later Tshalaia (1947) published some further observations of *E. moshkovskii*. The amoeba was re-isolated from places of the sewage disposal plant which were previously found to be infected with *E. moshkovskii*, and it was also found in several new parts of the sewer network of Moscow. Morphologically, similar amoebae were also isolated from two ponds. The organism was not observed directly in material from these ponds—as in the case of the sewage—but it developed when cultured. It was also isolated from a river in Minsk. In order to differentiate *E. moshkovskii* from *E. ranarum*, attempts were made to infect frog tadpoles with *E. moshkovskii* but without success.

Wright, Cram & Nolan (1942) reported the presence of an amoeba in sewage, which was possibly *E. moshkovskii*, but apart from noting that it resembled *E. histolytica* in structure, they did not make any further investigations.

E. moshkovskii has also been isolated from sewage in Brazil by Amaral & Azzi Leal (1949). A brief description of the morphology of the amoebae and cysts is given by these authors, and they conclude that it is morphologically indistinguishable from *E. histolytica*. In a later paper these authors (Azzi Leal & Amaral, 1950) report that they were not able to infect guinea-pigs with *E. moshkovskii* nor were they able to isolate *E. moshkovskii* from human faeces.

Gnezdilov (1947) reported its presence in the sewage of Leningrad, and a brief record of the occurrence of *E. moshkovskii* in sewage in London has already been published by me (Neal, 1950*a*).

In order to assist readers in understanding the terms used in sewage disposal, a brief description of the general method is given.

On entering the sewage disposal plant, the crude sewage is first run into sedimentation tanks where the suspended solid matter is allowed to sediment. The supernatant liquid is removed and sprinkled on to the surface of a thick bed of porous material (sprinkler beds) through which it slowly seeps; the soluble organic compounds it contains are converted by various aerobic organisms into harmless substances. The effluent from the sprinklers is generally discharged into a river. The sediment or sludge from the sedimentation tanks is first aerated

and then pumped into deep tanks where it remains for some time, during which it undergoes anaerobic digestion (digestion tanks). After digestion, the sludge may then be pumped out into shallow pans where it is allowed to dry. The details of the whole process may vary according to the presence of various industrial wastes in the incoming sewage.

II. MATERIAL, METHODS AND PRELIMINARY DATA

(*a*) *Isolation of* Entamoeba moshkovskii

Samples of crude sludge and digested sludge were collected from a sewage disposal plant in North London on 10 x. 1949. The crude sludge consisted of the sediment from the first sedimentation tank before the sewage had undergone any treatment, while the digested sludge was taken from a tank where the sediment had been digesting for some weeks. On direct examination, the crude sludge contained numerous Protozoa including various flagellates, ciliates, amoebae and entamoebic cysts of the *E. coli* and *E. histolytica* types; the digested sludge contained Protozoa in smaller numbers but including 4-nucleate entamoebic cysts.

Various types of media were inoculated with both samples and were incubated either at 37° C.—as was done by Tshalaia (1941)—or the tubes were left on the bench at room temperature. Although media inoculated with crude sludge and incubated at 37° C. grew various Protozoa and *Blastocystis* in primary culture, these subsequently died out on subculturing. The Protozoa present in the crude sludge grew in media left at room temperature and the cultures were subcultured every 1 or 2 days even though no amoebae were observed. *E. moshkovskii* was first observed in the sixth subculture in *hsm*+*S** medium. This strain of *E. moshkovskii*, known as *CS*†, was mixed with a flagellate and a few of the amoebae were infected with *Sphaerita*. *Sphaerita* disappeared quickly on continued subculture, but the flagellate, however, continued to flourish and was eliminated by treating the cysts of *Entamoeba moshkovskii* with N/20-hydrochloric acid for 60 min., after which this acid was neutralized with sodium bicarbonate and the cysts were incubated in *hsm*+*S* medium. A few amoebae were seen on the sixth day and thereafter grew and encysted with ease and in large numbers. This is strain *CXY*. The flagellates in strain *CS* were also eliminated after four subcultures had been grown at 37° C. during a period of 11 days (strain *CST*). During some experiments in attempting to grow *E. moshkovskii* aerobically it was discovered that this strain (*CST*) was contaminated with '*limax*' amoebae, but when the strain was examined 3 months later no '*limax*' amoebae were discovered nor have they been found since.

The digested sludge was treated in a similar manner to that adopted for the crude sludge. Cultures made with *hsm*+*S* medium at 37° C. were positive for *E. moshkovskii* in the fifth subculture and they appeared to be pure, but after this strain (*DSR*) had been subcultured at room temperature for 14 days a flagellate appeared; however, a second series of subcultures at 37° C. removed this contaminant which has not subsequently been detected. Thereafter the strain was kept at room temperature. The cultures inoculated with digested sludge and kept at room temperature grew various Protozoa and good growth of *E. moshkovskii* was found in *hsm*+*S* medium in the fourth subculture over 11 days (strain *DS*). The amoebae of this strain were accompanied by a small flagellate and small '*limax*' amoebae which were removed by a series of subcultures at 37° C. (strain *DP*).

It is noteworthy that the best medium for primary isolation was *hsm*+*S*, while the other media used (*hs*+*S* and *HShs*+*S*, Dobell & Laidlaw, 1926) were inferior in this respect.

The origin of the various strains is summarized in the diagram shown on p. 255, and methods used for isolating pure strains of *E. moshkovskii* are indicated in brackets.

(*b*) *The growth of* Entamoeba moshkovskii *in vitro and methods used for investigating the life cycle*

The following three media were used in this work: horse-serum diluted 1 : 8 parts with Ringer's solution buffered to pH 7·2 (medium abbreviated as *hs*), diluted horse-serum to which a solution of 'Marmite' was added (Jones, 1946; 'Marmite' is a soluble autolysed yeast extract: *hsm* medium) and a medium consisting of an inspissated horse-serum slant covered with Ringer's solution (*HSr* medium). The latter medium was used when it was thought necessary to increase the bacterial growth. A loopful of rice starch was added to all these media (*S*).

The Ringer's solution was that recommended by Dobell & Laidlaw (1926) for parasitic amoebae. Physiological saline solutions containing 0·5 and 0·85 % of sodium chloride were used, but gave no better results than those obtained with Ringer's solution.

Four pure strains have been extensively studied, *DP*, *DSR*, *CST* and *CXY*. All of these strains grew in a wide variety of different media, from the very rich media used for culturing *E. histolytica* (for example, *Ehs*+*S* or *HSre*+*S*, Dobell & Laidlaw, 1926) to dilute horse serum (*hs*+*S*). In the

* See below for description of media used for *E. moshkovskii*.

† The names of all strains isolated from the crude sludge begin with the letter *C*, while those isolated from digested sludge begin with the letter *D*.

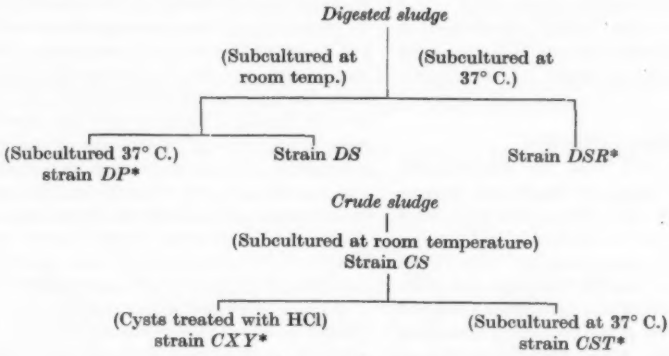
richer media, subculturing had to be more frequent, and although amoebae grew easily, encystation in these media was very poor or entirely absent. It was found that *DP* and *CXY* strains encysted best in *he*+*S* medium, while *hsm*+*S* medium was most suitable for strains *CST* and *DSR*. Wherever the medium is not specified in descriptions of the behaviour of these strains given below, it means that the optimum medium was used.

It was found that after inoculation of the appropriate medium the amoebae grew and multiplied, then after a certain time, which varied with different strains, the amoebae encysted in large numbers. Encystation usually coincided with the disappearance of all the solid rice starch.

kept in the refrigerator (4° C.) until it was necessary to produce some more cysts. The cysts were then hatched in fresh media at room temperature* and the amoebae grown until more cysts were formed. The new cysts were then placed in the refrigerator.

Experiments involving the determination of the time taken for the amoebae to pass through some stages of development, such as excystation, were carried out during the winter months at a constant temperature in an incubator at 24° C.

Growth of *E. moshkovskii in vitro* at 37° C. was very poor compared with that found in cultures maintained at 24° C. or room temperature. These experiments are described in detail later (see p. 264).



* Strains under cultivation at the time of writing.

The cysts did not hatch unless inoculated into fresh medium, although sometimes dilution of the old culture medium with water was sufficient to induce excystation in some cysts. Not all of the amoebae encysted, the proportion varying with different strains. The majority of amoebae of strains *DP* and *DSR* encysted and on casual inspection appeared to be free from amoebae, but careful examination revealed that a small number of amoebae were always present with the cysts. Only about 50% of amoebae of *CXY* encysted, while the proportion of encysting amoebae of strain *CST* gradually fell until after 30 months of cultivation *in vitro* this strain was virtually non-encysting, although the amoebae grew luxuriantly. Non-encysting strains may be induced to encyst by adding the bacterial flora from an encysting strain as was demonstrated by Dobell (1952) in the case of *E. histolytica*. Thus bacteria from *DP* strain induced amoebae of *CST* to encyst in large numbers.

It was found that cysts of *E. moshkovskii* will remain viable for 4–10 months (see below p. 262) provided the temperature remains at about 4° C. Therefore for maintenance of strains, cysts were

In order to study the excystation and metacystic development it was necessary to obtain cysts free from amoebae. Although it was found that exposure for 15 min. to N/20-hydrochloric acid would kill all amoebae, the hatching of cysts after acid treatment was irregular; the hatching was improved by seeding with favourable bacteria, but the numbers of amoebae produced were still not sufficiently plentiful for cytological study. Similarly, acriflavine at a concentration of 0·1% killed all amoebae, but with them many cysts. Evidently cysts of *E. moshkovskii* are much more sensitive to chemicals than are those of *E. histolytica*. Similar difficulties were encountered by Bishop (1937) with cultures of *E. aulostomi*.

E. moshkovskii, like all other species of *Entamoeba*, is an anaerobe, so a method utilizing this property was devised for exposing the amoebae to atmospheric oxygen. The sediment from the cultures was pipetted into conical flasks forming a layer not deeper than 1–2 mm. so as to expose the amoebae

* The room temperature varied from a maximum of 33° C. in the summer to a minimum of 17° C. during winter. There was a daily fluctuation of about 5° C.

to the air. When the flasks were kept at room temperature, exposure for 4 days was required to kill amoebae of *DP* strain, though up to 6 days was required by other strains. If the flasks containing the cysts and amoebae were placed in the refrigerator (4° C.), only 4 days' exposure was necessary to kill amoebae of all strains. The lower temperature also prevented the inocula from drying, which kills the cysts (see below, p. 262). As a routine method, therefore, cysts were treated for 4 days at 4° C.

A better method was to pipette the cysts on to the surface of a plate of non-nutrient agar, since the agar absorbed excess culture fluid thus ensuring maximum aeration while the cysts were kept moist. However, it was more tedious to recover the cysts from these plates than from the flasks.

Some of the cysts were also killed by treatment in the conical flasks, but sufficient numbers remained viable to allow cytological study of metacystic development.

(c) *Cytological technique*

The amoebae were well fixed by Schaudinn's, Bouin's, Carnoy's, or Zenker's fixatives; Schaudinn's fluid was mainly used. The cysts were more difficult to preserve, but Schaudinn's or Carnoy's fixatives were the best, while fixation with Bouin caused the cytoplasm to shrink from the cyst wall.

Heidenhain's haematoxylin gave well-stained preparations, but as the cysts generally contained large chromatoid bodies, which stained an intense black colour with this technique, one or more nuclei were generally obscured. For this reason phosphotungstic haematoxylin was preferred. This stain colours the chromatoid bodies a light coppery colour, and therefore enables the nuclei to be easily studied as these are deep blue in colour. The method was carried out either according to Dobell's (1942) original instructions, or to a modification described by Hoare (1949) as Cooper's method. The main difference between the methods is that the solution of haematoxylin in Dobell's method is used at room temperature, while in Cooper's modification the preparations are stained in a haematoxylin solution maintained at 37° C. As would be expected, the nuclei and cytoplasmic inclusions are more heavily stained by the latter method. Another great advantage of the phosphotungstic haematoxylin technique over Heidenhain's method is that no differentiation is required. The phosphotungstic haematoxylin method was used exclusively to stain smears prepared for the study of metacystic development, where the amoebae were sometimes rather scanty.

Other stains were tested in order to try and differentiate the nuclei from chromatoid bodies in the cysts. These included Hansen's iron trioxyhaematin which, according to Pantin (1946), stains only the nuclei; the results however were similar to those obtained with Heidenhain's haematoxylin. Chlorazol black was also tried, but gave very poor results.

Glycogen in the cytoplasm was stained with Lugol's iodine or with Best's carmine stain after fixation with Carnoy's fluid.

The figures were carefully drawn at a magnification of 2000 with the aid of a camera lucida.

III. MORPHOLOGY OF *ENTAMOEBA MOSHKOVSKII*

(a) *The amoebae of* Entamoeba moshkovskii

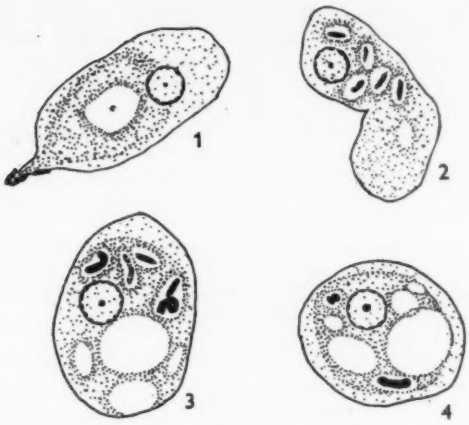
The living amoebae are very active organisms, their movement being of the so-called '*limax*' type by protrusion of one pseudopodium into which there is a constant streaming of endoplasm. The posterior end of the amoebae is frequently sticky and may have bacteria adhering to it (Fig. 1). The nucleus is only faintly visible so that the structure of the peripheral layer of chromatin is rarely seen. No contractile vacuole is present in normal amoebae; the question of the vacuolar structures appearing in amoebae immersed in hypotonic solutions is dealt with at greater length below (p. 263). The amoebae feed on grains of rice starch (Fig. 1) or bacteria (Fig. 2), but will not ingest erythrocytes.

As the amoebae are so active it is difficult to obtain precise measurements of their size; in an actively multiplying culture they may measure from 9 to 29 μ in diameter, but more generally are from 11 to 13 μ.

In fixed and stained amoebae, the nucleus appears as a spherical structure measuring about 2·5 to 4·0 μ in diameter, although its shape may be distorted by the pressure of ingested starch grains. The karyosome is a small, centrally situated granule or it may be composed of several smaller granules. Between the karyosome and nuclear membrane no other granules of chromatin are found. Occasionally there are a few strands connecting the karyosome with the nuclear membrane (so-called 'spoke radii'), but these stain much less intensely than the karyosome. The peripheral layer of chromatin is closely applied to the inner surface of the nuclear membrane and consists of a layer of fine granules, almost touching one another. Multinucleate amoebae were not observed in young healthy cultures.

(b) *The cysts of* Entamoeba moshkovskii

Before encysting, the amoeba ceases ingesting food, and commences to secrete glycogen; this is not at first concentrated in a definite vacuole, but is scattered diffusely throughout the endoplasm (Fig. 5). The amoeba then rounds off and secretes a cyst wall. Meanwhile, the glycogen becomes concentrated in a distinct vacuole. In the living cyst,

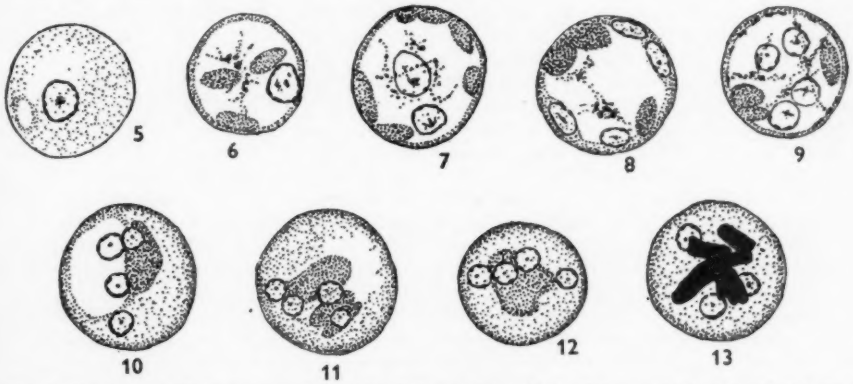


Figs. 1–4. Amoebae of *Entamoeba moshkovskii*. × 2000 (2 mm. = 1 μ). In all drawings of *E. moshkovskii* the preparations were fixed in Schaudinn's fluid and stained by phosphotungstic haematoxylin, unless otherwise stated.

Fig. 1. Amoeba from strain *DP* with ingested rice starch grain and bacteria adhering to the 'tail'.

Fig. 2. Amoeba from strain *DSR* with ingested bacteria.

Figs. 3, 4. Amoebae of strain *CXY* in tap water showing vacuolation of the cytoplasm. Fixed in Bouin's fluid and stained with Heidenhain's haematoxylin and eosin.



Figs. 5–13. Cystic stages of *Entamoeba moshkovskii*, all figures from strain *DP* except Fig. 13, which is from strain *CST*. × 2000 (2 mm. = 1 μ).

Fig. 5. Precystic amoeba, showing absence of cytoplasmic inclusions.

Fig. 6. Uninucleate cyst with large glycogen vacuole, chromatoid bodies and refractile granules.

Fig. 7. Binucleate cyst with large glycogen vacuole, chromatoid bodies and refractile granules.

Fig. 8. Quadrinucleate cyst showing all the nuclei pressed by the glycogen vacuole against the cyst wall. Nuclei seen in side view. Chromatoid bodies and refractile granules present.

Fig. 9. Similar stage as Fig. 8, but nuclei seen in face view.

Fig. 10. Quadrinucleate cyst with glycogen disappearing and maintaining a regular outline and a chromatoid body. Fixed in Zenker's fluid, stained in phosphotungstic haematoxylin.

Fig. 11. Similar to Fig. 10 but the glycogen vacuole shows an irregular edge. Fixed in Zenker's fluid, stained in phosphotungstic haematoxylin.

Fig. 12. Quadrinucleate cyst showing chromatoid body only. Fixed in Zenker's fluid, stained in phosphotungstic haematoxylin.

Fig. 13. Quadrinucleate cyst from strain *CST*, the fourth nucleus could be observed lying above the centre of the intensely stained chromatoid mass, but no details could be elucidated. Fixed in Schaudinn's fluid stained in Heidenhain's haematoxylin.

the wall measures about 1 μ in thickness and appears to consist of one layer; however, when the amoeba is excysting two layers are occasionally visible (Fig. 15).

The glycogen vacuole is very large, the cytoplasm of the cyst being limited to a very narrow rim at the periphery of the cyst (Figs. 6–9). It persists unchanged while nuclear division takes place and disappears within 5 days after the four nuclei are formed. As it decreases in size the entire edge to the vacuole may be maintained (Fig. 10), or it may become ragged (Fig. 11). In stained preparations after fixation with Schaudinn's or Zenker's fluid there are often some unstained but refractile granules present in the otherwise empty vacuole, the glycogen being dissolved out (Figs. 6–9). These granules cannot be seen in preparations stained by Best's carmine stains or Lugol's iodine where the glycogen is still intact, or in Bouin fixed material. Chromatoid bodies were also observed in the cysts of *E. moshkovskii*. These are present from the uninucleate stage and vary in number from 3 to 7 in each cyst. The chromatoid bodies are large and rather elongate with rounded ends (Figs. 6–13). When the glycogen vacuole is still present the chromatoid bodies lie round its edge, but when it is absorbed they occupy the centre of the cyst.

Owing to the presence of the glycogen vacuole, the nuclei in the 1-nucleate and 2-nucleate cysts are invariably distorted to an oval shape. The nucleus in the 1-nucleate cyst divides to form four nuclei in a manner similar to *E. histolytica*. Occasionally the four nuclei are of unequal size, generally with one large and one small nucleus, and two of equal size intermediate between the other nuclei (Fig. 41), although other combinations of different sizes may occur. These peculiar cysts are viable and hatch normally but their subsequent development has not been followed further than the first metacystic division. They occur in small numbers in all strains examined, but in greatest numbers in strain *CXY*. The structure of the cystic nuclei is similar to those of the amoebae except that they are smaller (generally 1·5–2·0 μ). Supernucleate cysts containing eight or sixteen nuclei have also been observed, and these are generally larger than normal cysts. These cysts hatch normally but it is not known whether they develop further.

The time taken for the cystic nuclei to complete their divisions is probably less than 24 hr. However, the exact time is difficult to determine accurately, since encystation is not undergone simultaneously by all amoebae in one particular subculture but is spread over several days. The time of onset of encystation varies with different strains. Amoebae of strain *DSR* start to encyst after 3–7 days of growth, and more than 90 % of the cysts are mature within 48 hr. of the commencement of encystation. Amoebae of strains *DP* and *CXY* begin to encyst after 6–8 days of growth, but while *DP* requires only 2–3 days for the majority of the cysts to ripen, *CXY* strain requires 3–5 days. Strain *CST* formed mature cysts within 14 days. In good encysting strains, cysts are formed in large clumps; each of these contain several hundred cysts, the total number in each subculture amounting to thousands.

Cysts of *E. moshkovskii* are generally spherical in shape and vary in size from 7·0 to 16·8 μ. Mature cysts from strains *CST*, *CXY*, *DSR* and *DP* were measured after being coloured by Lugol's iodine solution. These measurements are recorded in Table 1.

Table 1. *The size of mature cysts of* Entamoeba moshkovskii

Strain	No. measured	Range (μ)	Mean ± standard error (μ)
DP	200	6·9 to 13·9	10·30 ± 0·10
DSR	200	8·3 to 12·5	10·45 ± 0·07
CST	200	8·3 to 15·3	11·19 ± 0·08
CXY	200	8·3 to 16·7	11·82 ± 0·13

When plotted graphically, these data gave unimodal polygons; the measurements of two strains, *DP* and *CXY*, are treated in this manner in Fig. 14.

(c) *The excystation and metacystic development of* Entamoeba moshkovskii

The following description of the excystation and metacystic development of *E. moshkovskii* is based on a study of material from strain *DP*. Observations made with other strains, *DSR* and *CXY*, showed that these followed the same pattern as *DP*. In order to present a uniform series of illustrations, only amoebae from strain *DP* are depicted (see Figs. 15–40).

A cyst commencing to hatch can be recognized by a number of changes in the cytoplasm. All food reserves disappear, the four nuclei become clumped together and a number of small vacuoles and granules appear at the centre of the cytoplasm. The cytoplasm shrinks away from the wall forming a gap of about 2 μ between the amoeba and the cyst wall (Figs. 15, 16). The amoeba then begins to move inside the cyst, pseudopodia being pressed against the wall until it is perforated by a small hole through which a small portion of the cytoplasm is first protruded and then withdrawn (Fig. 17). This 'see-sawing' motion is continued repeatedly with larger portions of the cytoplasm emerging each time until the amoeba escapes completely out of its cyst (Fig. 18). The empty cysts remain spherical and apparently intact for a short time, but they are eventually invaded by bacteria and after about 24 hr. disappear.

The wall in all mature cysts and in the majority of hatching individuals appears to be composed of a single layer. Occasionally a thin flexible inner wall can be observed in hatching cysts. It is possible that it is present in all cysts, but does not become detached from the rigid outer wall. In fixed and stained material it is difficult to observe (Fig. 15).

divides and is followed by a cytoplasmic division. The formation of the daughter amoebae takes place in such a way that each amoeba contains one daughter nucleus, together with a variable number of the nuclei derived from the cyst (termed cystic nuclei). The different kinds of nuclei are distinguished by their relative size, the cystic nuclei being larger than the recently divided nuclei. It

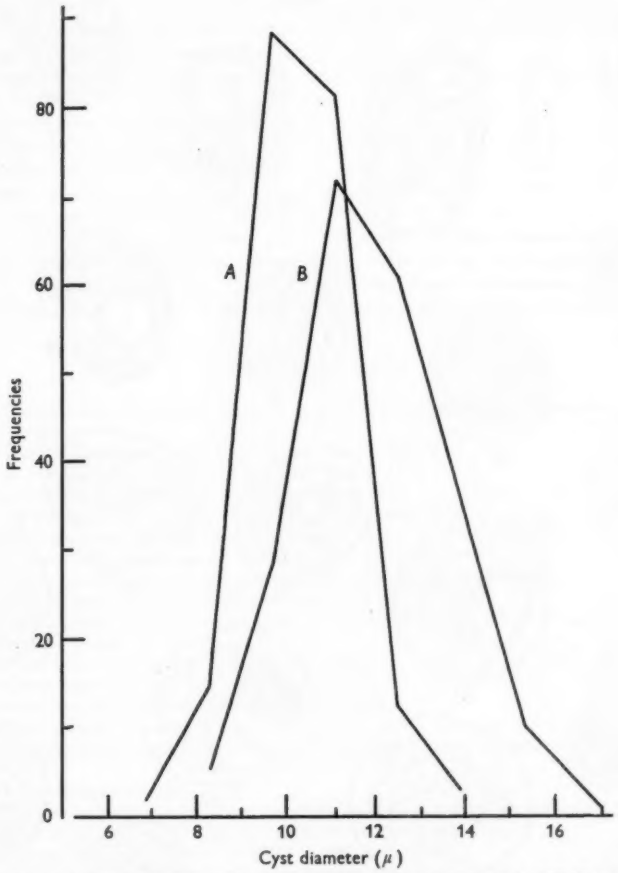


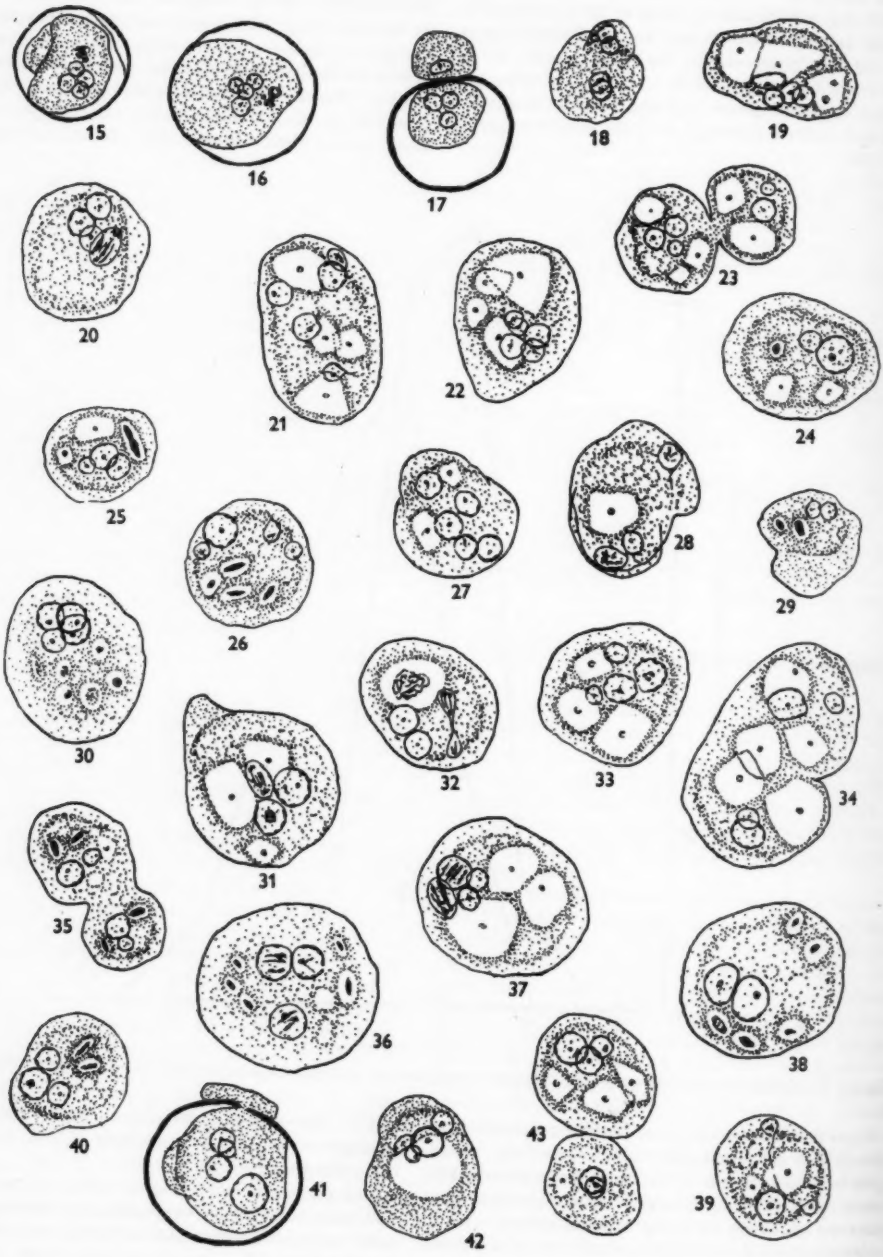
Fig. 14. Polygons showing the cysts of *Entamoeba moshkovskii*. A, cysts from strain *DP*; B, cysts from strain *CXY*.

Cysts first showed signs of hatching 2–3 hr. after inoculation into suitable culture medium. Most cysts hatched after 5–8 hr., but a few continued to hatch up to 12 hr. Exceptionally, one cyst was observed hatching 18 hr. after inoculation.

After hatching, the multinucleate amoeba ingests rice starch or bacteria (Fig. 19) and commences to increase in size. It then undergoes a series of nuclear and cytoplasmic divisions, each nucleus can be seen therefore, that as a result of the cytoplasmic divisions the amoebae will not contain more than four nuclei.

The proportion of amoebae with one, two, three and four nuclei was determined at different times after inoculation of a culture tube with *E. moshkovskii* cysts. This data is given in Table 2.

These figures show that the majority of amoebae were uninucleate after 23 hr. and the metacystic



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development was completed. A few amoebae were observed with more than four nuclei and these were probably amoebae which had failed to divide after the division of a cystic nucleus (see Figs. 22 and 27). The numbers of these amoebae were, however, small and are omitted from Table 2.

The sequence of nuclear and cytoplasmic divisions follows two main patterns. In the first, one of the four cystic nuclei divides (Figs. 20–22), and the following cytoplasmic division (Fig. 23) gives rise to two amoebae, one with two cystic nuclei and one daughter nucleus (Fig. 25), the other with one cystic nucleus and one daughter nucleus (Fig. 24). These amoebae then continue to ingest food and to grow. The cystic nuclei in these amoebae ultimately also divide, and after corresponding cytoplasmic divisions form uninucleate amoebae (Figs. 26–29).

In the second case, the cytoplasmic division which follows soon after the division of the first cystic nucleus takes place in such a way that one of the daughter amoebae contains three cystic nuclei (Fig. 31). The actual cytoplasmic division of this type, however, has not been seen with certainty, but amoebae with this nuclear constitution are quite common in the early stages of metacystic development of *E. moshkovskii*. One of the cystic nuclei in these amoebae divides (Figs. 31, 32), followed by a cytoplasmic division into two amoebae, each containing one cystic and one daughter nucleus (Figs. 34, 35), though occasionally

Table 2. *The proportion of amoebae of* DP *strain with different numbers of nuclei present in cultures of different ages*

Duration of incubation (hr.)	No. counted	Percentage of amoebae: Four nucleate	Three nucleate	Two nucleate	One nucleate
4–8	157	95·6	1·9	2·6	0
12	400	56·25	11·0	11·75	20·0
16	486	24·1	22·8	14·8	38·3
18	555	13·3	18·2	15·1	52·3
20	528	11·4	13·3	14·0	61·0
23	500	3·8	5·8	7·2	83·2
48	250	0·4	0	1·6	98·0

Legends for Figs. 15–43.

Figs. 15–43. Excystation and metacystic development in *Entamoeba moshkovskii*. Figs. 15–40 are from strain *DP*: Figs. 41–43 from strain *CXY*.

Fig. 15. Cyst commencing to hatch, showing inner layer of cyst wall, 2 hr.
Fig. 16. Cyst hatching, with amoeba retracted from cyst wall, 6 hr.
Fig. 17. Amoeba emerging from cyst through small aperature, 4 hr.
Fig. 18. Newly hatched amoeba, without any cytoplasmic inclusions, 4 hr.
Fig. 19. Recently hatched amoeba with ingested rice starch, 12 hr.
Fig. 20. Amoeba with four cystic nuclei one of which is dividing, 16 hr.
Fig. 21. Amoeba with three cystic and two daughter nuclei, recently divided, 16 hr.
Fig. 22. Amoeba with three cystic and two daughter nuclei, 16 hr.
Fig. 23. Dividing amoeba, forming one amoeba with one cystic and one daughter nuclei the other with two cystic and one daughter nuclei, 12 hr.
Fig. 24. Amoeba with one cystic and one daughter nuclei, 16 hr.
Fig. 25. Amoeba with two cystic and one daughter nuclei, 16 hr.
Fig. 26. Amoeba with one cystic and three daughter nuclei, 16 hr.
Fig. 27. Amoeba with five daughter nuclei, 23 hr.
Fig. 28. Dividing amoeba forming one amoeba with two daughter nuclei, the other with one daughter nucleus, 23 hr.
Fig. 29. Amoeba with two daughter nuclei, 16 hr.
Fig. 30. Amoeba with three cystic nuclei and one daughter nucleus. A small amoeba was lying adjacent to this individual with a corresponding small daughter nucleus, 16 hr.
Fig. 31. Amoeba with three cystic nuclei one of which is dividing, 20 hr.
Fig. 32. Similar stage to Fig. 31, with nuclear division further advanced, 16 hr.
Fig. 33. Amoeba with two cystic and two daughter nuclei, 16 hr.
Fig. 34. Large amoeba dividing to form two amoebae each with one cystic and one daughter nuclei, 16 hr.
Fig. 35. Similar stage to Fig. 34 with cytoplasmic division further advanced, 18 hr.
Fig. 36. Amoeba with three cystic nuclei, two of which are dividing, 16 hr.
Fig. 37. Amoeba with two cystic nuclei undergoing division and two daughter nuclei, 16 hr.
Fig. 38. Amoeba with two cystic nuclei, 18 hr.
Fig. 39. Amoeba with one cystic nucleus and two recently divided daughter nuclei, 18 hr.
Fig. 40. Amoeba with one cystic and two daughter nuclei, 16 hr.
Fig. 41. Cyst with unequal sized nuclei commencing to hatch (strain *CXY*), 6 hr.
Fig. 42. Newly hatched amoeba with nuclei of unequal size (strain *CXY*), 6 hr.
Fig. 43. Recently divided amoeba, one with two cystic and one daughter nuclei, the other with one cystic and one daughter nuclei (strain *CXY*), 12 hr.

the cytoplasm does not divide (Fig. 33). The subsequent divisions of these 2-nucleate amoebae cannot of course be distinguished from amoebae with identical nuclear constitutions formed by the previous method (see Fig. 23).

One amoeba was observed which contained three cystic nuclei and one daughter nucleus (Fig. 30), and lying nearby (though not drawn) was a small uninucleate amoeba, which suggests that the cytoplasmic division following the primary cystic nuclear division, sometimes produce an amoeba containing all nuclei except one daughter nucleus.

In spite of the fact that occasionally amoebae were seen with two cystic nuclei dividing simultaneously (Figs. 36 and 37), amoebae with five or more nuclei (Fig. 27) were extremely rare.

Amoebae were sometimes observed with two cystic nuclei (Fig. 38), one of which divided forming amoebae with one cystic and two daughter nuclei (Figs. 39, 40).

As described above (p. 258), a variable proportion of mature cysts showed nuclei of unequal size. Cysts of this kind were observed in *DP* strain, but were relatively uncommon. On the other hand, strain *CXY* formed a large proportion of these peculiar cysts. These cysts are viable and hatch in the same manner as other cysts (Figs. 41, 42). Moreover, after the division of one cystic nucleus (Fig. 43) a cytoplasmic division occurs in the same manner as is described above for normal cysts (see Fig. 23). It seems probable, therefore, that the cysts with nuclei of unequal size will develop in the same manner as normal cysts.

IV. *IN VITRO* EXPERIMENTS

(*a*) *The viability of* Entamoeba moshkovskii *cysts*

(i) *Length of survival of the cysts*

In spite of the difficulties involved in obtaining cysts free from amoebae (see above, p. 255), it was found that hydrochloric acid-treated cysts will survive at room temperature for at least 87 days; occasionally they hatch after being kept for 100 days, but never after longer periods. This probably represents the minimum time of survival since, as described above (p. 255), a variable proportion of cysts die after hydrochloric acid treatment.

It was easier to test the viability of cysts when kept at 4° C. since amoebae will not live at this temperature longer than about 2 months.

The data in Table 3 show that cysts of strains *DP* and *DSR* remained viable at 4° C. up to 143 days. After this time the cysts were occasionally viable up to 237 days in the case of strain *DP*, and up to 313 days for *DSR* cysts, but after this time all cysts were dead. Cultures of strain *CST* were always positive from cysts kept up to 117 days and occasionally up to 190 days, but not after this time. Cysts of strain *CXY* were always positive up to 300 days. This is the longest time yet tested for this strain.

When it was found that the cysts of two strains remained viable for long periods at 4° C. it was thought that perhaps some cysts may have hatched, the amoebae developed normally and re-encysted. However, the cysts of these strains (*CXY* and *DSR*) were examined repeatedly during the sojourn in the refrigerator, but no evidence of excystation was ever found. Also cysts of *CXY* and *DSR* which had been kept at 4° C. for 300 and 272 days, respectively, were actually observed hatching after inoculation into fresh medium.

Table 3. *The duration of viability of cysts of* Entamoeba moshkovskii *at* 4° C.

	Strains		
	DP	*DSR*	*CST*
Time (days)	143+	143+	117+
	147−	150−	120−
	157+	170+	125+
	168+	175+	132 to 143−
	179−	178−	146+
	237+	182 to 313+	157+
	256−	337−	170−
			190+
			337−

On one occasion the temperature of the refrigerator was sufficiently low to cause the medium in the culture tube to freeze solid. However, after thawing, the cysts were found to be viable.

(ii) *Effect of drying on the cysts*

The sediment from a culture of *DP* strain containing thousands of mature cysts was pipetted into two dry sterile test-tubes. At the same time a small portion was inoculated into a tube of *HSr+S* medium. The sediment in the test-tubes was allowed to dry by evaporation, which was completed by the sixth day. About 10 ml. of *hsm* medium was then added to the dry sediment, together with a loopful of rice starch. Although bacteria grew well, no amoebae were observed, showing that the cysts were dead whereas the controls showed good growth. A similar experiment was carried out with *CST* cysts, with identical results.

It is possible that the concentrated culture fluid present during evaporation might have killed the cysts by virtue of its high osmotic pressure or presence of toxic substances. A third experiment was accordingly carried out with *CST* cysts, but

these were washed three times with Ringer's solution and finally with sterile water before being allowed to dry. However, again no amoebae were observed after the culture medium was added to the dry sediment. It therefore seems certain that cysts of *E. moshkovskii* cannot withstand drying.

(*b*) *The development of 'contractile vacuoles' in amoebae of* Entamoeba moshkovskii

As mentioned above (p. 252), Tshalaia (1941) observed that when the amoebae were placed in hypotonic solutions, 'contractile vacuoles' appeared. As this observation is of great interest, these experiments were repeated.

Amoebae of strain *CXY* were transferred to sterile tap water at room temperature and examined after various intervals. Within 24 hr. the endoplasm had developed numerous small spherical vacuoles about 1 μ in diameter. These vacuoles coalesced and formed larger vacuoles until they reached a maximum size of about 4–5 μ in diameter. Only a single large vacuole was present at any one time (Figs. 3, 4). The vacuolar contents were homogeneous and contained no solid particles. Where the amoebae were moving actively in a typical fashion, the largest vacuole appeared to pass towards the posterior end of the organism, where it discharged its contents through a small temporary pore. The vacuoles generally emptied at the posterior region but occasionally lateral discharge was observed. Sometimes the vacuole only partly discharged its contents. The formation of the vacuoles was also observed with amoebae of strains *DP* and *CST*.

After about 24 hr. all the amoebae had developed these vacuoles in the endoplasm. The amoebae were active and moved in a typical fashion; however, if they were observed for longer periods it was found that they eventually ceased moving and died. The exact time when all amoebae were dead depended in the first instance on the degree of dilution of the inoculum with the hypotonic liquid. For example, some amoebae of *DP* strain were still viable after 7 days in half-strength Ringer's solution where the inoculum was diluted 1 : 44, while all amoebae were dead after 4 days when the dilution was 1 : 88. In another experiment, where the dilution was 1 : 160, all amoebae were dead after 2 days.

One experiment showed that the formation of vacuoles was reversible. Amoebae of *CXY* strain were transferred to tap water. After 3 days many amoebae were moribund, but some were still moving actively, and all—so far as could be determined microscopically—had developed 'contractile vacuoles'. These were then inoculated into *hsm* + *S* medium. After 7 days, many normal *E. moshkovskii* amoebae, without 'contractile vacuoles', were observed.

Vacuolation of the endoplasm is a common degenerative phenomenon in parasitic entamoebae, but this interpretation cannot completely explain the appearance of these vacuoles in *E. moshkovskii* amoebae, owing to the regularity of their formation and discharge. It seems likely that the amoeba is trying to eliminate excess water by means of these vacuoles, but it cannot continue indefinitely and eventually dies. Other causes, of course, may contribute to the death of the amoeba.

The contracting vacuoles in *E. moshkovskii* cannot be considered as true contractile vacuoles as they are only found in amoebae placed in abnormal habitats where they eventually die.

(*c*) *Attempts to grow* Entamoeba moshkovskii *under aerobic conditions*

As *E. moshkovskii* has been found under conditions which indicate that it was a free-living species, it is obviously of great interest to determine whether the amoebae are capable of aerobic growth.

Amoebae and cysts of *DP* strain were inoculated on to the surface of Petri plates of Musgrave and Clegg's agar (Kudo, 1946) to which 3 ml. of boiled tap water were added. The plates were kept at room temperature. One live amoeba was observed in scrapings from the agar surface of one plate 3 days later, but none was seen after this time. Cysts, microscopically normal, were observed up to the sixth day.

In another experiment, the same medium was inoculated with amoebae of *CST* strain, but again no growth of amoebae was observed.

Plates of Musgrave and Clegg's agar were also inoculated with amoebae from strain *DS*. It was known that this strain was not pure but was contaminated with one of the small '*limax*' species. It was therefore not surprising to find that many of the latter were growing but no amoebae of *E. moshkovskii* were observed.

In a final attempt to grow *E. moshkovskii* aerobically, the method devised by Singh (1950) was used. A loopful of *Aerobacter* sp. (Singh's strain) was placed on non-nutrient agar and the bacteria was inoculated with cysts of *DSR* strain. Twelve days later the culture was examined but no amoebae were observed, although many cysts, morphologically normal, were still present. The cysts were viable as was shown by subinoculation into *hs* + *S* medium.

These experiments show that amoebae of *E. moshkovskii* do not grow when cultivated by methods previously found to be suitable for certain aerobic amoebae. The cysts survive for at least 12 days but do not hatch under aerobic conditions. It should be pointed out here that in the other media described above (p. 254), *E. moshkovskii* grows under anaerobic conditions. This may easily be shown by the bleaching of methylene blue,

previously incorporated into the medium, when anaerobiosis is established.

(*d*) *Growth of* Entamoeba moshkovskii *at* 37° C.

Tshalaia (1941) found that *E. moshkovskii* grew very well when cultivated at 37° C.—amoebae were found for 2–3 weeks and cysts after 1–1½ months; while Azzi Leal & Amaral (1950) found that growth of *E. moshkovskii* from digested sludge at temperatures from 15 to 37° C. 'was exuberant within 24–48 hr.'.

In view of the possible relationship with *E. histolytica* it was decided to investigate this problem more fully.

The first attempts to grow the London strains of *E. moshkovskii* at 37° C. was made with strain *CK*. This strain was prepared by adding the bacterial flora from a strain of *E. histolytica* (*K*.28c) to *E. moshkovskii* strain *CST* (see also below, p. 265). It was found, in agreement with the previous reports, that the amoebae of this strain grew abundantly at 37° C. in *hsm*+*S* medium, the amoebae always remained viable for 10 days and often for longer periods. No cysts were observed; however, this strain would not encyst even at 24° C.

Experiments were then carried out to determine if cysts would develop at 37° C. Cysts of strain *DP* were inoculated into *hs*+*S*, *hsm*+*S* and *HSr*+*S* media, but cultures were never obtained and only dead cysts were observed microscopically, while those of *DSR* sometimes hatched in *hsm*+*S* medium but not in *hs*+*S* or *HSr*+*S* media. Cysts of strain *CXY* never developed in *hs*+*S* medium, but cultures were always obtained with *hsm*+*S* and *HSr*+*S* media. The growth of amoebae of *CXY* in these media was always poor (+ as compared with + + + at 24° C.), while the absence of amoebic growth in *hs*+*S* was probably due to the very poor bacterial growth, macroscopically the cultures appeared sterile.

The cysts used for these experiments were freed from amoebae by storage at 4° C. (the cysts had been stored for 112–229 days) and their viability was demonstrated by inoculating a small number into suitable media maintained at 24° C. In all cases, a good growth of amoebae was obtained.

Cysts of strains *DP*, *DSR* and *CXY* were hatched at 24° C. and the amoebae then transferred to 37° C. Amoebae of *DP* died out within 3 days in all three culture media, those of *DSR* grew well (+ + +) at first but then decreased in numbers (±) in either *hsm*+*S* or *HSr*+*S* media, while those of *CXY* grew slightly better (+) than *DSR*. The amoebae grew regularly in small numbers, but cysts were not observed to be formed. However, the amoebae encysted normally if they were transferred from 37 to 24° C.

All these results are briefly summarized in Table 4.

In attempting to evaluate the significance of these results, it is difficult to eliminate the effects of the bacterial flora, as growth at a higher temperature will upset the balance of growth between the different bacteria. This latter factor probably accounts for the inability of strain *DP* to grow at a temperature of 37° C. However, it seems correct to deduce that in the presence of a favourable flora and with suitable media, limited excystation and growth of *E. moshkovskii* can be obtained. The most important result is that encystation was not observed. This latter observation shows that *E. moshkovskii* is adapted to growth at lower temperatures.

Table 4. *Growth of* Entamoeba moshkovksii *at* 37° C.

Strain	Excystation	Growth of amoebae	Encystation
DP	–	–	–
DSR	±	±	–
CXY	+	+	–
CK	–	+ + +	–*

* *CK* would not encyst, even at room temperature.

V. ATTEMPTS TO INFECT VARIOUS ANIMALS WITH *ENTAMOEBA MOSHKOVSKII*

The observations recorded above show that *E. moshkovskii* is closely related to the *histolytica* group of species of *Entamoeba*. As it is possible that *E. moshkovskii* may in fact be an *Entamoeba* from an amphibian which has found its way to a favourable habitat, experiments were carried out to determine the infectivity to tadpoles. Similarly, in order to differentiate it from *E. histolytica*, experiments with rats were carried out in order to discover if *E. moshkovskii* was infective to an animal which is susceptible to infection with *E. histolytica*.

(*a*) *Attempts to infect rats*

Ten young rats (aged 3–4 weeks) were inoculated intracaecally with cultures of amoebae of *DP* strain. It was found that two out of three animals examined 5 hr. after inoculation were positive; in one of the positive animals amoebae were observed still with rice starch ingested from the culture. One out of two rats examined 2½ days later were positive. The amoebae observed in this animal appeared to be degenerate, but grew when inoculated into *hsm*+*S* medium. No rats were found positive after this time.

Five rats were inoculated orally with cysts of *DP*, nine with *CXY* cysts and three with *DSR* cysts. All these rats were negative when examined 2–6 days after inoculation, and subculture of caecal material in *hsm*+*S* medium was also negative.

The material used for inoculation in the above experiments was obtained from cultures grown at room temperature, but in an attempt to increase the infectivity of *E. moshkovskii* to rats, amoebae from *CST* were grown at 37° C. In addition, the total bacterial flora from a strain of *E. histolytica* (K.28c), which is known to be infective to rats, was added so as to enable *E. moshkovskii* to become accustomed to bacteria which live at 37° C. After growth for 5 days at 37° C. amoebae of this strain (now designated *CK*) were inoculated intracaecally into five young rats. Two animals were killed after 5 days and one of these was positive. In material obtained directly from the caecum the amoebae microscopically appeared to be degenerate, but they grew normally in culture (*hsm*+*S* medium). No amoebae were observed in the remaining three animals killed 7 days after inoculation either microscopically or culturally.

The observation that one rat inoculated with strain *CK* was found infected 5 days after inoculation suggested that further growth of strain *CK in vitro* at 37° C. might increase the infectivity to rats. Accordingly, strain *CK* was maintained at 37° C. for another 6 weeks, and the experiment repeated. This time, six rats were inoculated intracaecally with amoebae of *CK* strain, two were killed 2 days after inoculation, the remainder after 7 days. All these animals were negative both microscopically and culturally.

These results show that amoebae inoculated intracaecally may survive in the caeca of rats for a short time—up to 5 days—but invariably die out after this time. Cysts administered orally do not establish an infection.

(*b*) *Attempts to infect frog tadpoles*

Tadpoles of the common frog (*Rana temporaria*) were obtained free from intestinal Protozoa by artificial fertilization of the eggs of sexually mature frogs, thereafter rearing the larvae with sterile precautions, according to the method described by Dobell (1918). The strains of *E. moshkovskii* used for these experiments had been maintained *in vitro* for 17 months.

Cysts of *DP* strain were washed and fed to six tadpoles (10 days old). These began to eat the sediment immediately it was offered to them. Three of the tadpoles fed with *DP* cysts were killed and examined 4 days later, and the remainder were killed and examined 6 days after feeding with cysts. They were all negative for amoebae, although a few cysts were observed in the intestine.

This experiment was repeated with cysts of *DP*, *DSR* and *CST* strains. Cysts of each strain were washed three times, then fed to three groups of six tadpoles each (22 days old). Three tadpoles of each group were killed 6 days after feeding with cysts and the remainder were examined 14 days after feeding with cysts. No amoebae were observed in the intestines of these animals, although cysts were occasionally seen. These were still viable as was shown by subinoculation into *HSr*+*S* medium.

A small number of the cysts of each strain fed to tadpoles were inoculated into suitable culture media in order to test their viability. Good growth of amoebae was always obtained.

These experiments show that cysts of *E. moshkovskii* pass through the intestine of tadpoles (larvae of *Rana temporaria*), but they do not hatch and establish an infection.

(*c*) *Attempts to infect* Salamandra maculosa *larvae*

Four larvae were delivered alive from the uterus of a sexually mature female *S. maculosa*. Two of these were kept as controls, whilst the other two were fed with chopped earthworms previously injected with a suspension of cysts of *DP* strain. The larvae were 7 days old when fed with cysts. The viability of the cysts used for feeding to the larvae was demonstrated by the good growth obtained in *hsm*+*S* medium.

The larvae were killed and examined 13 days later. No amoebae were observed microscopically in the recta of any animal. One culture made in *hsm*+*S* medium from the larvae fed with *DP* cysts was positive; however, this result was probably due to viable cysts present in the intestine, as observed with *Rana temporaria* larvae, rather than to an established infection.

VI. DISCUSSION

The morphology of *Entamoeba moshkovskii*, described above, agrees with that reported for this species by Tshalaia (1941) and Amaral & Azzi Leal (1949). A morphological feature, which however was not observed by previous workers with the sewage amoeba, is the presence during encystation of a persistent glycogen vacuole. This character has been noted in other amoebae, *E. ranarum* (Sanders, 1931), *E. pyrrhogaster* (Lobeck, 1940), and *E. knowlesi* (Rodhain & Hoof, 1947). It is interesting to note that all these workers were dealing with *in vitro* cultures in which the amoebae were well supplied with starch. Taliaferro & Fisher (1926) studied cultures of *E. ranarum*, but unlike Sanders, did not observe a persistent glycogen vacuole; however, their culture medium was not supplemented by rice starch. It is therefore possible that the persistence of glycogen may merely be due to the availability of abundant starch to the amoebae. On the other hand, other species of *Entamoeba*, when cultivated *in vitro* with starch, do not form

this large amount of food reserve during encystation, for example *E. histolytica*, *E. terrapinae* (Sanders & Cleveland, 1930), *Entamoeba* sp. from a Ganges turtle (Knowles & Das Gupta, 1930) and *E. aulostomi* (Bishop, 1932).

Further observations are required in order to determine whether this feature may be used to distinguish certain species of *Entamoeba*.

The formation of cysts with four nuclei of unequal size was observed by previous workers with *E. moshkovskii* and also in the present work. Similar abnormalities have been recorded by Bishop (1932, 1937) for *E. aulostomi*, and the present author has also seen them in *E. histolytica* cysts. They are also figured in *E. histolytica* by Epstein (1941). The formation of unequal-sized nuclei in the case of *E. moshkovskii* may be due to the pressure exerted on dividing nuclei by the large glycogen mass, but this explanation cannot apply in the case of *E. aulostomi* where the glycogen disappears before the nuclei complete their divisions (Bishop, 1932). Bishop (1937) was uncertain whether these peculiar cysts actually hatch, but the observations described above show that in *E. moshkovskii* they hatch in the same manner as normal cysts, and the first metacystic division is similar to that observed for amoebae derived from typical cysts.

The description of the hatching of cysts and the metacystic development of amoebae confirms Tshalaia's work and amplifies many details. It closely resembles that described for *E. aulostomi* by Bishop (1937), differing only in minor details from that of *E. histolytica* described by Dobell (1928).

Experiments with the London strains of *E. moshkovskii* showed that it will not grow under aerobic conditions. Growth only occurred when anaerobic conditions were present, as shown by the bleaching of methylene blue when this is added to the medium. Azzi Leal & Amaral (1950), on the other hand, consider that *E. moshkovskii* will not grow anaerobically, but these authors do not present any convincing evidence on this point.

It does not seem likely that *E. moshkovskii* will infect man since rats (see experiments described above), kittens (Tshalaia, 1941), and guinea-pigs (Azzi Leal & Amaral, 1950) have been found to be refractory to experimental infection with this species of *Entamoeba*. Faeces from cases of human carriers of *histolytica*-like amoebae were examined by culturing at room temperature for the presence of *E. moshkovskii*, but without success. Azzi Leal & Amaral (1950) were also unable to detect *E. moshkovskii* in human faeces by the same method.

We turn now to the classification of *E. moshkovskii*, which obviously belongs to that group of species of *Entamoeba* which form 4-nucleate cysts and produce from the cyst, by successive nuclear and cytoplasmic divisions, eight 1-nucleate amoebae. Only four species of this group are fairly completely known, *E. histolytica*, *E. ranarum*, *E. invadens* and *E. aulostomi*. *E. moshkovskii* is morphologically very similar to all four species, but it differs from *E. histolytica*, *E. invadens* and *E. aulostomi* in the presence of a persistent glycogen mass (although this feature is of doubtful taxonomic value, see above), but the metacystic development resembles most closely that of *E. aulostomi* (that of *E. ranarum* has not yet been fully described). *E. moshkovskii* differs physiologically from *E. histolytica* in the low optimum temperature of growth, and from *E. ranarum* by its inability to infect frog tadpoles.

The morphological differences between *E. moshkovskii* and other species are however very slight, in fact, the typical trophic amoeba and the mature cyst could not be distinguished morphologically from any of the four species mentioned above, or from a number of less well-known species* of this group of *Entamoeba*.

E. moshkovskii has been found in sewage plants in widely scattered parts of the world, viz. Moscow (Tshalaia, 1941, 1947), Leningrad (Gnezdilov, 1947), São Paulo, Brazil (Amaral & Azzi Leal, 1949), California (Wright, Cram & Nolan, 1942) and finally London. In view of its wide geographical distribution, it is possible that future work may show conclusively (for comments on Tshalaia's (1947) work, see below) that it occurs in places other than sewage disposal plants. The obligate anaerobic growth of *E. moshkovskii*, and its association with large numbers of bacteria, suggests its possible occurrence in other places where there is a high concentration of decomposing organic material. Moreover, *E. moshkovskii* must be regarded as a polysaprobic organism, since in addition to the inhibitory effect of oxygen on growth, dilution of the culture medium causes the death of the amoebae in spite of attempts by the amoebae to eliminate excess water by means of contracting vacuoles (see Tshalaia, 1941, and above, p. 263).

Consideration of the means of dispersal of cysts of *E. moshkovskii* in sewage plants shows that the amoeba is living in a habitat from which it cannot reach new sites of growth. The sewage sludge, after remaining in the digestion tanks for some time, is pumped out into shallow pans and is allowed to dry; this operation will kill the majority of cysts. Cysts may be discharged, however, in the effluents from the sprinkler-beds as shown by the observation of Gordon (1941), who found *histolytica*-like cysts in this effluent. It will be remembered that this

* The term species is used in this discussion in its most restricted sense. Many of the so-called species of *Entamoeba* may in fact be identical with one another, as has been previously noted in another connexion (Neal, 1950*b*).

amoeba was found by Tshalaia (1947) in many parts of the sewer-network of Moscow, and also that *E. moshkovskii* was isolated in the present work from crude sewage as it reached the disposal plant. These facts show that *E. moshkovskii* must be continually pouring into the sewage disposal plants from outside sources.

Therefore circumstantial evidence suggests strongly that the sewage disposal plant may not be the primary habitat of *E. moshkovskii*.

In this connexion, it is interesting to note that Tshalaia (1947) has isolated amoebae which she considers morphologically identical with *E. moshkovskii* from two ponds in Moscow and a river in Minsk. However, as pointed out above, morphological evidence is not sufficient to differentiate it with certainty from amoebae also likely to occur in these places, for instance *E. ranarum* or *E. aulostomi*. In the same paper Tshalaia (1947) describes some experiments suggesting that the sewage amoeba will not infect frog tadpoles (species of frog not quoted), but she does not indicate with certainty whether the strains of *E. moshkovskii* used were those obtained from the ponds, river or sewage.

It should also be pointed out here, that it is incorrect to argue as she does, that the pollution of natural collections of water (ponds, etc.) with sewage will deter the presence of frogs. Savage (1939) showed for *Rana temporaria* that the presence of sewage actually attracts spawning frogs.

In conclusion it is interesting to speculate whether *E. moshkovskii* was actually seen, though not recognized, by Lackey (1925) during his study of the fauna of digestion tanks (= Imhov tanks) of sewage disposal plants in New Jersey, U.S.A. He writes: 'Finally an examination of the sludge was made by cultural methods....Some unknown forms, which have never been seen in the tanks, also developed...for example, a small very lobose amoeba without vacuoles.'

VII. SUMMARY

1. *Entamoeba moshkovskii* was isolated from a sewage plant in North London.

2. The morphology and metacystic development is similar to that of *E. histolytica* and other *histolytica*-like amoebae, but with differences in minor details.

3. The cysts of *E. moshkovskii* remain viable when kept at 4° C. for periods up to 10 months providing they are not allowed to dry.

4. If the amoebae are placed in hypotonic solution, the amoebae attempt to eliminate excess water by means of contracting vacuoles, but they eventually die.

5. Experiments have shown that *E. moshkovskii* is an anaerobe.

6. The optimum temperature for growth is about 24° C. as encystation does not occur at 37° C.

7. *E. moshkovskii* is not infective to rats or to larvae of the common frog (*Rana temporaria*) and *Salamandra maculosa*.

I should like to thank Mr Frank Lee, Engineer to the Urban District Council of Enfield, and Mr Toomer, Manager of the Sewage Works, for the samples of sewage sludge. I am also indebted to Dr R. M. Savage who collected the adult frogs, and Mr P. Bradley for the *Salamandra maculosa* larvae. Dr C. A. Hoare kindly translated the papers of the Russian workers and criticized the manuscript.

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TWO NEW MITES FROM SOUTH AFRICAN MAMMALS (LISTROPHORIDAE, SARCOPTIFORMES)

By R. F. LAWRENCE

Natal Museum, Pietermaritzburg, South Africa

(With 4 Figures in the Text)

Subfamily Labidocarpinae Gunther

Genus *Labidocarpus* Trouessart

Labidocarpus cristatus n.sp. (Fig. 1)

Types. Four mature females (NM. 5728) from *Myotis tricolor*, Town Bush Cave, Pietermaritzburg, March 1952.

immediately distinguished, is the crest in the anterior half of the dorsal surface of the body (Fig. 1*a*), composed of minute serrations (three or four to one of the normal annulations). This crest comes to an abrupt termination and the usual type of annulation, passing right across the body, takes its place (Fig. 1*a*). Spination as in Fig. 1*a*, that of

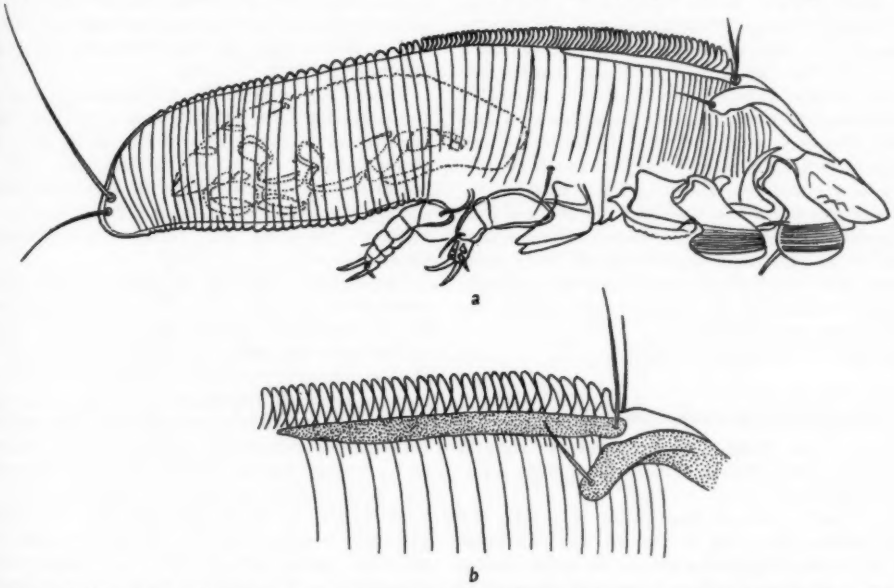

Fig. 1. *Labidocarpus cristatus* n.sp. *a*, mature female with hexapod larva seen through the body wall; *b*, enlarged portion of dorsal crest just behind the head.

Female (Fig. 1*a*). The species differs markedly from *L. natalensis* Lawrence (1952, p. 140) in the long, slender parallel-sided body. The chitinization of the upper surface of the head is characterized by a long narrow rod, separate from the remaining chitinous structures of the head region and passing backwards for a considerable distance just below the dorsal margin; this rod is absent in other species of *Labidocarpus*. The most striking feature, however, by means of which the species can be the legs not differing from *L. natalensis*. A small spine (absent in *L. natalensis*) below the pair at the posterior apex of the head dorsally. Number of body annulations about fifty-six, the crest consisting of about sixty smaller divisions. The type female and another specimen contains a hexapod embryo with the head directed posteriorly.

Dimensions. Length of body 328 μ, greatest depth 85 μ.

These specimens, as in other known species of

Labidocarpus, occur in the body fur on the dorsal and ventral surfaces of the body in the neighbourhood of the shoulders of the bat. They are much smaller and rather more active than the females of *Alabidocarpus calcaratus* Lawrence, of which specimens were taken on the nose vibrissae of the same host specimen. Unfortunately no males were found.

Subfamily MYOCOPTINAE Gunther

Taterobia n.g.

Male. Propodosoma short, triangular, with a single triangular scute in the middle and a much smaller subquadrate plate at each basal angle, the main scute with a very stout, long seta at each basal angle, its anterior extension not covering the mouthparts, hysterosoma almost entirely covered by a single large scute. Tarsi I, II and IV with small but distinct caruncles, those of IV greatly modified and with quite different apical structures. Legs I and II moderate, II slightly stouter than I, III greatly enlarged, IV small; tarsi of legs I and II, and to a less extent the tibiae, with a strong pointed triangular process ventrally as in certain genera of Analgesidae (feather-mites). Caudal suckers and caudal process strongly developed, the latter well chitinized, with three long and one much shorter seta laterally and posteriorly. All legs clearly consisting of five segments, the palpi of two or three.

Copulatory nymph with the dorsal propodosomal scutes as in the male, remainder of body soft-skinned, with annulations; four long setae ventrally at posterior apex of abdomen. Legs normal, the anterior ones as in the male.

Genotype. T. longisetosa n.sp.

Taterobia longisetosa n.sp. (Figs. 2–4)

Types. One male, one copulatory nymph (NM. 5750), from *Tatera brantsi*, Kosi Bay, Zululand, July 1952.

Dorsal surface as in description of genus, the propodosoma spined as in Fig. 3*a*, the two large basal setae reaching backwards as far as the caudal suckers; hysterosoma with a large continuous scute finely punctured as in the propodosomal scute, except along its anterior margin just posterior to the dividing suture; two setae near the anterolateral angle, the anterior considerably longer than the posterior one.

Ventral surface as in Fig. 2, the male genital opening ill-defined and not chitinized; the setae at the extremity and sides of the caudal process long, the second (from the posterior apex) the longest, about three-fourths the total length of body, the fourth the shortest (Fig. 3*b*, *e*).

Legs. Leg II spined as in Fig. 3*c*, seen from below, tarsus III as in Fig. 3*d*, seen from below.

Pedipalps closely apposed to the chelicerae and appearing to be to some extent modified for clasping a hair, consisting of two distinct segments, the second fairly slender; in addition, there is what appears to be a short basal segment at the insertion of the appendage, but its outlines are very indistinct.

Copulatory nymph. I hesitate to describe this as a female, as its whole appearance indicates immaturity; it is considerably smaller than the male.

Dorsal surface. Propodosoma not differing from that of the male, except that the two large basal setae are considerably shorter, not passing backwards far beyond coxa IV; hysterosoma entirely without a scute, soft and annulated, with two lateral setae; two minute setae near its anterior margin in the middle about the same width apart as the large spines of the propodosoma; another similar pair at the posterior level of coxa IV, a considerably longer pair halfway between coxa IV and the posterior apex of the abdomen, these more laterally situated.

Ventral surface as in Fig. 4, the anus situated near the posterior apex of abdomen. Anterior legs in general resembling those of the male, II a little stouter than I, IV a little stouter than III, the posterior legs differing from those of the male in having a stout conical dagger-like process at the inferior apices of the tarsi, directed straight forwards.

Dimensions. Length of body: male, 665 μ; copulatory nymph, 500 μ. Width of body: male, 377 μ; copulatory nymph 252 μ.

The male was taken in copula with the nymph, the two being joined back to back with the caudal plate of the male passing over the posterior end of the nymph, as has been observed in other species of *Listrophorid* mites such as *Schizocoptes conjugatus* and *Listrophorus leporicolus*. The resemblance of this male to various genera of feather-mite parasites in the modified posterior legs and caudal process is very striking. The ventral processes of the anterior tarsi, which are the distinguishing characters of Trouessart's Section II (Analgeseae) of the Analgesinae (1915, p. 218), are extraordinarily similar to those of the species under consideration, and it is difficult to understand how such resemblances can have come about by convergence.

I have accordingly taken into account the possibility of this mite being a straggler from a bird, although I think it would be unlikely that a highly specialized feather-mite would be found in the fur of a mammal or could live very long in such an environment. With this in view I have examined feather-mites from the Rock martin (*Ptyonoprogne fuligula*) which was the most plentiful species of bird in the locality where the host Gerbille was

captured. The mites found on this bird, however, represented by a male, female and a number of nymphs, are very different from the species described in this paper from *Tatera brantsi*.

mole. This genus, described by me from *Chrysospalax* (*Amblysomus*) *trevelyani* (1951, p. 127), was taken on Museum skins; the type specimens are unfortunately incomplete but there is enough to

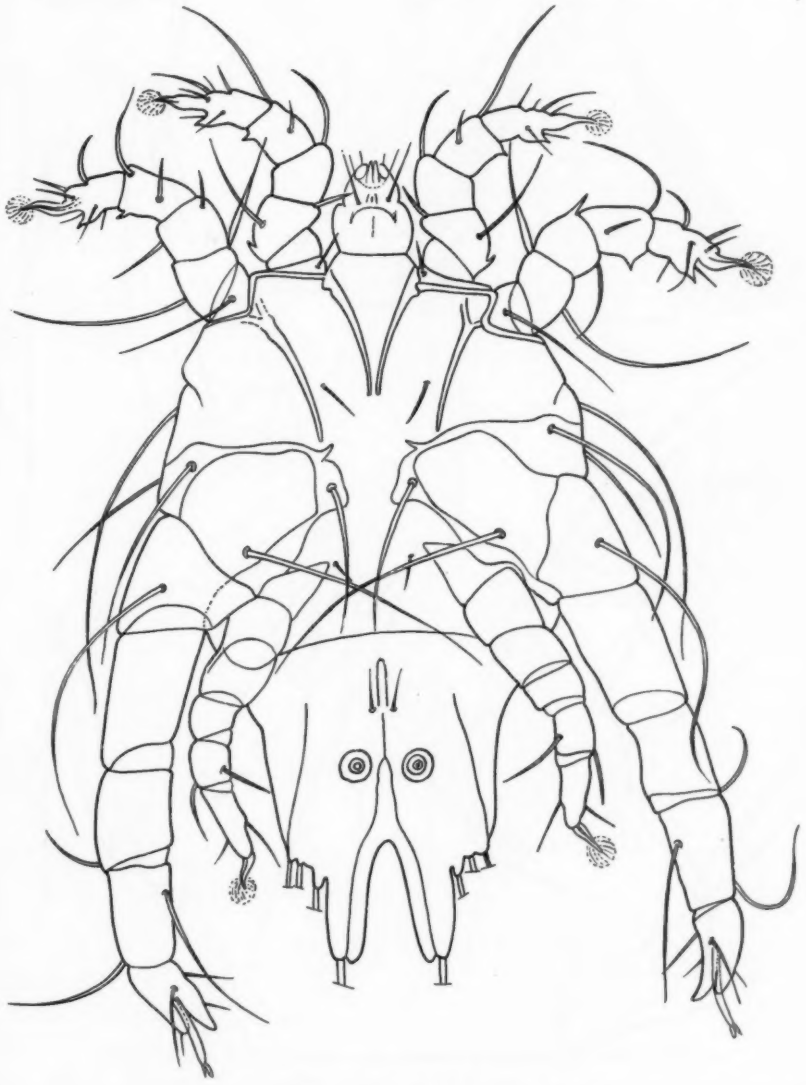

Fig. 2. *Taterobia longisetosa* n.sp. ♂; ventral surface.

Another reason for doubting that this mite is an Analgesid living temporarily on a mammal, is the occurrence of another rather similar genus, *Chrysocoptes*, on the South African giant golden

show clearly that the sharp ventral processes of the anterior legs also occur in this genus.

The genera occurring on mammals which appear to belong to Gunther's subfamily Myocoptinae are

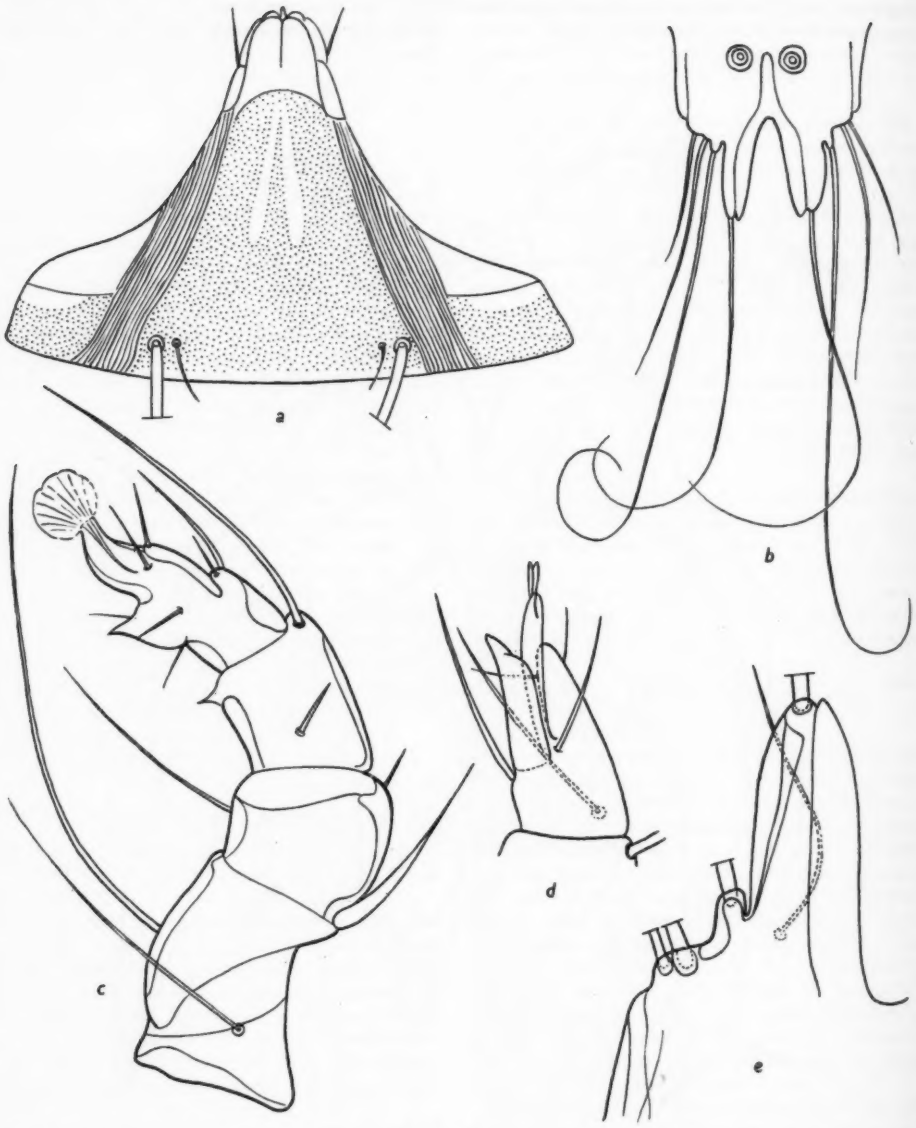

Fig. 3. *Taterobia longisetosa* n.sp. ♂; *a*, dorsal surface of propodosoma; *b*, ventral surface of caudal plate to show comparative length of the setae; *c*, ventral surface of leg II; *d*, apex of leg III enlarged (ventral surface); *e*, left half of caudal process enlarged (ventral surface).

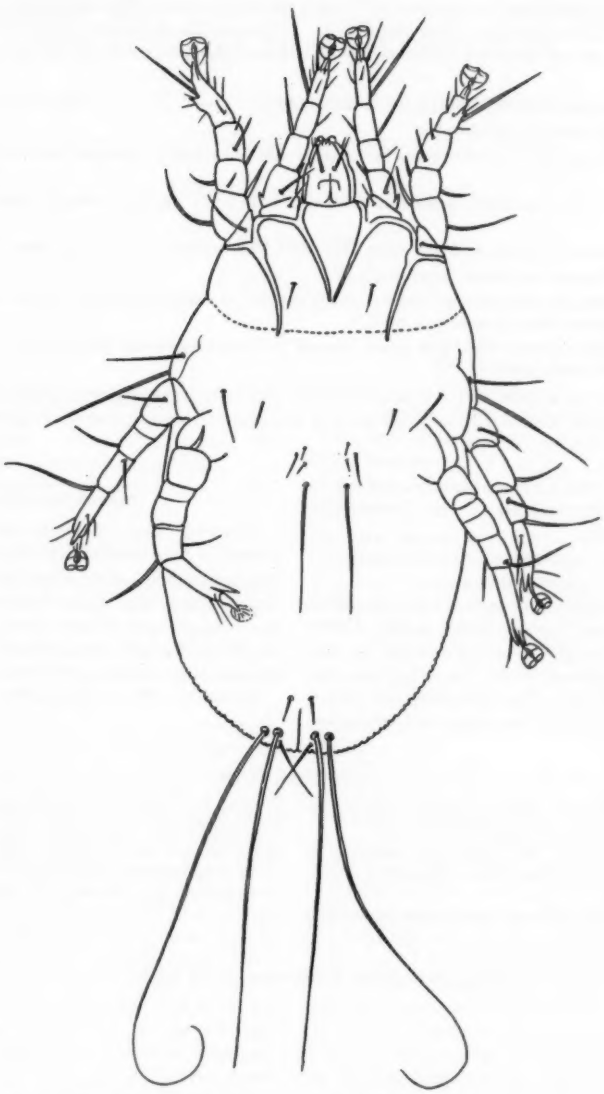

Fig. 4. *Taterobia longisetosa* n.sp.; ventral surface of copulatory nymph.

all found on rodents of the families Sciuridae, Graphiuridae, Cricetidae and Muridae, except *Chrysocoptes*, which lives on a member of the peculiar family Chrysochloridae; this family is regarded by some recent workers as forming an independent order, the Chrysochloridea, which is probably more nearly related to the Insectivora than to the Rodentia.

These genera can be provisionally distinguished by means of the key shown above.

The species *Myocoptes kalrai* described by Radford from an Egyptian Gerbille (1946, p. 232) differs in many respect from the genus *Myocoptes* in the restricted sense represented by its genotype, *M. musculinus* (C. L. Koch). Radford does not figure the dorsal surface, but the characters of the ventral surface are alone sufficient to distinguish it from the known genera of Myocoptinae. I therefore propose the name *Neomyocoptes* for the reception of this species, and also in all probability for *Myocoptes tenax* Michael, with the following characters.

1. None of the legs modified as claspers, no dorsal scutes. female of *Cryptocoptes* Lawrence
– Some of the legs modified as claspers. 2
2. Legs III and IV modified in both sexes but similar and not greatly enlarged; male without suckers. *Trichoecius* Canestrini
– Legs III and IV very dissimilar in the male and either III or IV greatly enlarged; male with or without suckers. 3
3. Leg IV of male much enlarged, larger than III; male with suckers. *Myocoptes* Claparède (sens. strict.)
– Leg III of male much enlarged, larger than IV. 4
4. Male without suckers; anterior legs without sharp ventral processes; posterior legs of both sexes without caruncles; caudal process of male not bifid. *Neomyocoptes* n.g.
– Male with suckers; anterior legs with sharp ventral processes; posterior legs with or without caruncles; caudal process of male bifid. 5
5. Propodosoma in the middle with a single triangular scute; posterior legs with caruncles. *Taterobia* n.g.
– Propodosoma in the middle with separate anterior and posterior scutes; posterior legs without caruncles. *Chrysocoptes* Lawrence

Neomyocoptes n.g.

Anterior legs of both sexes with caruncles, posterior legs without; posterior legs in both sexes much modified. Male with leg III much enlarged, much larger than IV; male with no suckers but with a caudal process which is not bifid. Female with leg III only a little larger than IV, both considerably stouter than the anterior legs.

Genotype. Myocoptes kalrai Radford.

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EGG SIZES AND DIFFERENTIAL EGG COUNTS IN RELATION TO SHEEP NEMATODES

By G. CUNLIFFE and H. D. CROFTON

Department of Zoology, The University of Bristol

(With 6 Figures in the Text)

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I. INTRODUCTION

Worm egg counts have been used for the assessment of nematode infections of sheep for many years. Different methods have been used, but recently the Stoll method (Stoll, 1930) and the McMaster Slide (Gordon & Whitlock, 1939) technique have almost entirely superseded the others.

The McMaster technique is the best method, although it suffers from the slight disadvantage that eggs of *Trichuris ovis* are difficult to detect because they do not float in the salt solution used. In most methods partial differentiation of the eggs into species can be made because the eggs of *Trichuris*, *Nematodirus* and *Strongyloides* can be easily recognized. This recognition is based upon the fact that these eggs differ considerably in size and/or shape from each other, and also from the main body of trichostrongyle eggs found in sheep faeces. For many purposes, however, the proportion of eggs of *Trichuris*, *Nematodirus* and *Strongyloides* is relatively unimportant, and knowledge of the numbers of the more pathogenic species is required. In such cases, faeces-charcoal cultures are prepared and the resulting larvae identified. This latter method has numerous faults, and most workers who have employed it extensively will have been conscious of difficulties in standardizing the technique. The recovery of different species of larvae from cultures seems to be dependent upon the amount of moisture in the culture jars and the temperature and time of incubation. Thus, apart from the method being slow and laborious, differential counts do not necessarily give a good estimate of the species of worm eggs in the sample. Moreover, a relatively highly skilled operator is necessary for the identification of larvae.

The problems of diagnosis of species by examination of eggs has been discussed by many authors—see Ransom (1911), Mönnig (1934), Ross & Gordon (1936), Freeborn & Stewart (1937), Shorb (1939, 1940), Tetley (1941*a*, *b*, 1949) and Kates & Shorb (1943).

Shorb (1939) indicated how various species of eggs might be identified in mixed nematode infections of ruminants. He used fifteen diagnostic characters, of which only two referred to egg dimensions. The other characters used are difficult to determine and necessitate decisions being made with only arbitrary standards as a guide. Both Shorb (1940) and Tetley (1941*b*) showed how eggs of species of certain genera could be distinguished from other members of the genus. Tetley (1941*a*) showed how eggs of *Haemonchus contortus* could be identified in mixed populations. Obviously these workers' methods are only applicable under special conditions. Tetley (1949) used a graphical method to indicate how in certain cases an egg could be assigned to a species.

After consideration of the previous work it was decided that any method which attempted to identify individual eggs in a mixed sample was impracticable and that egg differentiation could only be made on a probability basis with large samples. Measurements of the long and short axes of eggs seemed to be the only easily determined

statistics which could be obtained efficiently. The measurements of Shorb (1939) could not be used because, first, it was not known if the dimensions of eggs of species in the United States were exactly the same as those in Great Britain; secondly, Shorb did not give details of the frequency distribution of dimensions; thirdly, Shorb did not describe his method of measurement, and although he gave means and standard deviations the degree of accuracy of his measurements could not be assessed. It was decided to measure eggs of different species, using a method which could be standardized.

II. METHOD

One hundred mature females of each of the following species were picked at random from collections of worms taken from a number of sheep: *Haemonchus contortus, Cooperia curticei, Trichostrongylus axei, T. vitrinus, Bunostomum trigonocephalum, Chabertia ovina, Oesophagostomum venulosum* and *Ostertagia* spp. It is to be noted that while the females of species of *Ostertagia* could not be distinguished, almost all the eggs from females of this genus were probably *O. circumcincta* because the number of males of *O. trifurcata* and *O. ostertagi* occurring in the samples was negligible.

With each species of worm the procedure was the same. One egg was dissected from each worm and mounted in sufficient water to prevent distortion when a cover-glass was added. So far as could be ascertained, the egg nearest the vulva was used in each case.

Measurements were made by using a microscope (× 15 ocular, 16 mm. objective) fitted with a camera lucida, the image being measured with an ivory scale accurately subdivided into millimetres. The equipment was calibrated by using a stage micrometer slide, the microscope being adjusted until 0·1 mm. on the micrometer coincided with 40 mm. of the ivory scale seen as an image in the camera lucida. Measurements were thus made with 2·5 μ as a unit (= 1 mm. on ivory scale), and each dimension was expressed as $n \times 2{\cdot}5\,\mu$, this being the mid-point of a linear interval

$$[(n \times 2{\cdot}5) - 1{\cdot}25]\mu - [(n \times 2{\cdot}5) + 1{\cdot}25]\mu.$$

Records were kept for each individual egg.

III. EGG DIMENSIONS

(1) *Measurements of eggs*

Table 1 summarizes the data collected. For comparison the appropriate measurements made by Shorb (1939) are given. It will be noted that eggs varied in length from 62·5 μ (*H. contortus* minimum) to 125 μ (*T. vitrinus* maximum) and in width from 30 μ (*C. curticei* and *T. axei* minimum) to 60 μ (*O. venulosum* maximum). The standard deviations for length were all of the same order (3·87–6·68) as were those for width (1·8–3·74).

(2) *Size distribution of eggs*

The individual measurements of eggs of different species are plotted in Figs. 1–5. It will be noted

Table 1. *Egg dimensions*

(Measurements of the eggs of different species. Measurements by Shorb (1939) are given for comparison.)

Genus and species	No. of eggs	Length (μ)				Width (μ)			
		Max.	Min.	Mean	S.D.	Max.	Min.	Mean	S.D.
Haemonchus contortus	100	90	62·5	73·3	4·59	50	40	44·3	2·19
Cooperia curticei	100	87·5	67·5	77·5	3·87	40	30	35·6	1·80
Trichostrongylus axei	100	107·5	75	86·5	5·47	47·5	30	39·5	3·34
T. vitrinus	100	125	85	101·1	6·68	55	37·5	46·7	3·74
Bunostomum trigonocephalum	100	102·5	80	91·9	5·13	50	35	45·6	2·97
Chabertia ovina	100	100	77·5	90·6	4·39	57·5	45	51·0	2·40
Ostertagia spp.*	100	105	82·5	92·4	4·49	57·5	42·5	49·5	3·59
Oesophagostomum venulosum†	100	120	85	101·2	6·55	60	45	51·1	2·86
Shorb's data									
Haemonchus contortus	100	82	65	74	2·6	46	39	43·4	1·4
Cooperia curticei	100	82	70	76·8	2·7	41	35	37·7	0·9
Trichostrongylus axei	52	92	79	84·6	3·6	41	31	36	2·1
T. vitrinus	100	118	93	103·2	1·7	52	41	45	2·3
Bunostomum trigonocephalum	100	97	82	89·2	3·3	57	47	51	1·9
Chabertia ovina	100	100	83	90·9	2·8	59	47	53·2	2·0
Ostertagia spp.*	100	103	85	94	3·9	56	44	48·2	2·5
Oesophagostomum venulosum†	100	105	85	92·7	3·8	59	47	52·2	1·5†

* Mainly if not entirely *O. circumcincta*.

† Shorb's figures for *Oesophagostomum venulosum* from goats.

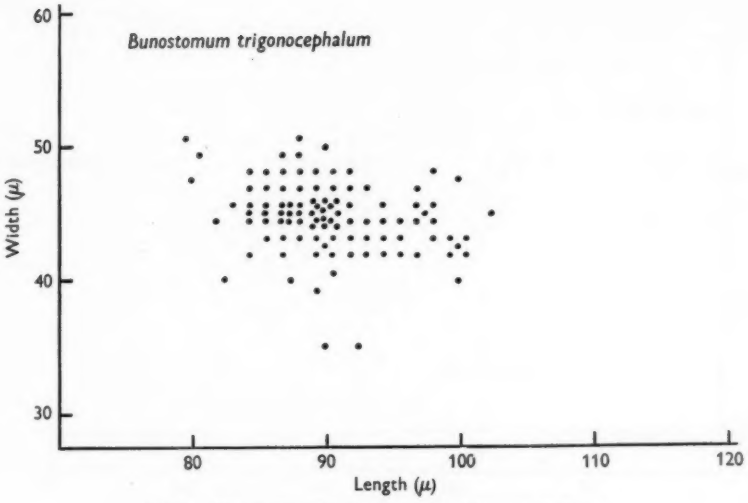

Fig. 1. Measurements of eggs of *B. trigonocephalum*

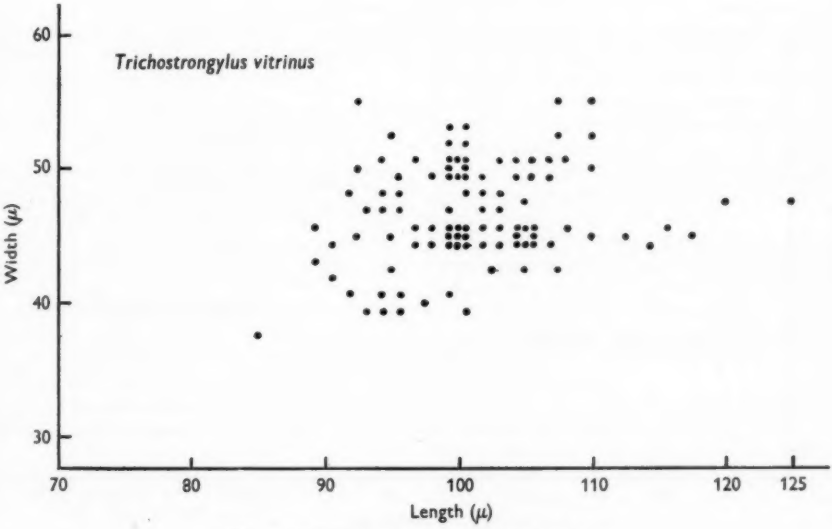

Fig. 2. Measurements of eggs of *T. vitrinus*

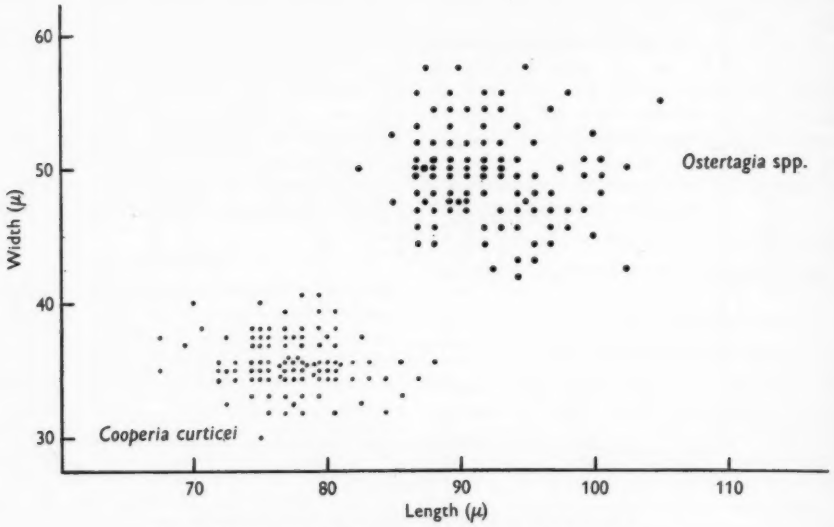

Fig. 3. Measurements of eggs of *C. curticei* and *Ostertagia* spp.

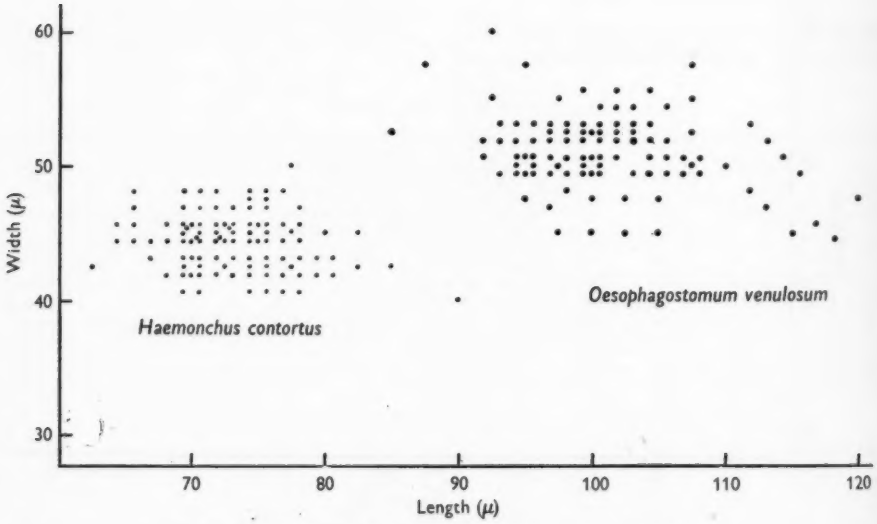

Fig. 4. Measurements of eggs of *H. contortus* and *O. venulosum*

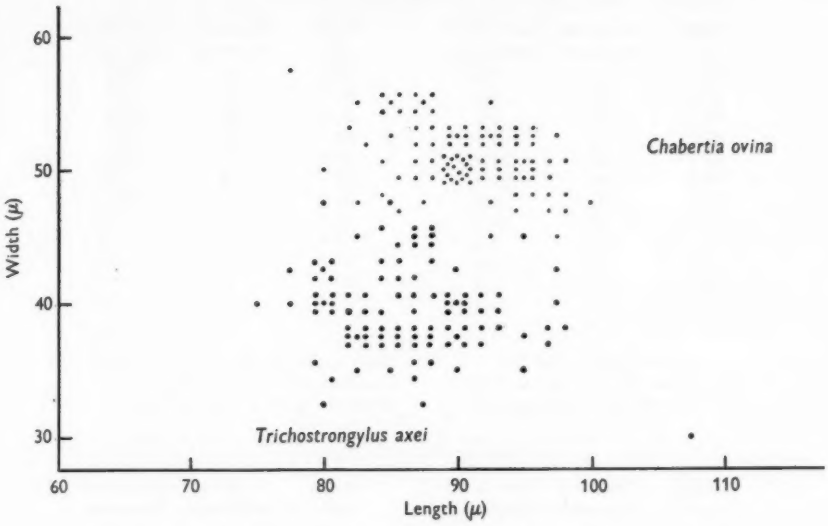

Fig. 5. Measurements of eggs of *T. axei* and *C. ovina*

that the form of distribution is similar to that given by Tetley (1941*a*, *b*, 1949) for *H. contortus* and *N. filicollis* and *N. spathiger*. The general pattern of distribution of eggs in a mixed sample containing equal numbers of each species can be seen by superimposing the graphs on common axes.

(3) *Frequency distributions of the linear dimensions of the eggs*

Tables 2–9 show the frequencies of length and width distribution for the different species. In each table is given the frequencies calculated on the assumption that the distribution is normal. The goodness of fit of the calculated with the observed frequencies is given.

It will be seen that P_{χ^2} varies between 0·1 and 0·7 if *T. vitrinus* width is excluded. In the case of *T. vitrinus* the calculated frequency is significantly different from the observed one ($P_{\chi^2} < 0{\cdot}01$), and for this reason the observed figures for the width of this species are used in later calculations.

IV. DISCUSSION OF RESULTS OF EGG MEASUREMENT

There is fairly close agreement between the measurements of the eggs and the figures quoted by Shorb (1939). In every case, however, the range of length and width given by Shorb is smaller (Table 1).

It is probable that this discrepancy may be due to the fact that only one egg per worm was measured in the present work, whereas it is obvious from Shorb's description that he measured several eggs from each worm. The authors have noted that when only the first egg from the vulva of each worm is measured the range is slightly greater, presumably because a larger sample of worms has been used. The main discrepancy between Shorb's figures and our own is in the case of *Oesophagostomum venulosum*, but it must be noted that in this case Shorb used eggs from worms which had been obtained from goats.

The size distribution of eggs (Figs. 1–5) is of the same general pattern described by Tetley (1941*a*, *b*). This author assumes that the distribution of length and width of *Haemonchus* is bivariate (1941*a*). It is clear from Figs. 1–5 that there is little or no correlation between length and width in any of the species considered.

In all species except *T. vitrinus* the distribution of length and width is approximately normal. Tetley assumes that this is so for *H. contortus*, *N. spathiger* and *N. filicollis* by his construction of areas calculated from his value for means and standard deviations. The exceptional case (*T. vitrinus* width) will be investigated more fully later, but for the present purpose the actual figures have been used. It is to be noted that the main difference between the observed and calculated distribution is in the central part of the distribution, the 'tails' showing fair agreement.

Table 2. *Haemonchus contortus*

Length (μ)	Observed frequency	Calculated frequency	Width (μ)	Observed frequency	Calculated frequency
60	0	0·44	37·5	0	0·6
62·5	1	1·5	40	8	8·9
65	6	3·4	42·5	33	31·4
67·5	5	9·8	45	41	41
70	24	16·9	47·5	17	16·2
72·5	18	21·0	50	1	1·9
75	22	20·2	52·5	0	0·07
77·5	15	14·1			
80	5	7·6			
82·5	2	3·0			
85	1	0·9			
87·5	0	0·2			
90	1	0			

Mean length, 73·275μ
Standard deviation, 4·592μ
Goodness of fit, $P_{\chi^2}=0{\cdot}2$

Mean width, 44·25μ
Standard deviation, 2·19μ
Goodness of fit, $P_{\chi^2}=0{\cdot}5$–$0{\cdot}7$

Table 3. *Cooperia curticei*

Length (μ)	Observed frequency	Calculated frequency	Width (μ)	Observed frequency	Calculated frequency
65	0	0·19	27·5	0	0·01
67·5	2	1·0	30	1	0·8
70	3	4·3	32·5	14	14·4
72·5	9	11·4	35	52	48·9
75	22	21·0	37·5	26	31·9
77·5	29	25·5	40	7	3·9
80	23	20·3	42·5	0	0·08
82·5	6	11·2			
85	4	4·0			
87·5	2	1·0			
90	0	0·17			

Mean length, 77·45μ
Standard deviation, 3·87μ
Goodness of fit, $P_{\chi^2}=0{\cdot}3$–$0{\cdot}5$

Mean width, 35·6μ
Standard deviation, 1·8μ
Goodness of fit, $P_{\chi^2}=0{\cdot}3$–$0{\cdot}5$

Table 4. *Trichostrongylus axei*

Length (μ)	Observed frequency	Calculated frequency	Width (μ)	Observed frequency	Calculated frequency
72·5	0	1	27·5	0	0·07
75	1	3·1	30	1	0·6
77·5	2	4·8	32·5	2	3·5
80	16	9·1	35	9	12·2
82·5	13	13·7	37·5	31	24·6
85	17	17·5	40	30	29·0
87·5	21	17·9	42·5	15	20·0
90	14	14·6	45	10	8·0
92·5	7	10·2	47·5	2	1·9
95	3	5·6	50	0	0·3
97·5	5	2·5			
100	0	0·89			
102·5	0	0·28			
105	0	0·06			
107·5	1	0·01			

Mean length, 86·525μ
Standard deviation, 5·47μ
Goodness of fit, $P_{\chi^2}=0{\cdot}1$

Mean width, 39·525μ
Standard deviation, 3·34μ
Goodness of fit, $P_{\chi^2}=0{\cdot}3$–$0{\cdot}5$

Table 5. *Trichostrongylus vitrinus*

Length (μ)	Observed frequency	Calculated frequency	Width (μ)	Observed frequency	Calculated frequency
82·5	0	0·47	35	0	0·26
85	1	0·82	37·5	1	1·44
87·5	0	1·93	40	9	5·65
90	4	3·72	42·5	6	14·42
92·5	7	6·41	45	36	23·85
95	13	9·92	47·5	15	25·61
97·5	7	12·67	50	23	17·65
100	25	14·86	52·5	7	8·18
102·5	11	14·37	55	3	2·42
105	14	12·77	57·5	0	0·52
107·5	8	9·35			
110	4	6·28			
112·5	1	3·49			
115	2	1·78			
117·5	1	0·75			
120	1	0·28			
122·5	0	0·08			
125	1	0·04			

Mean length, 101·125 μ
Standard deviation, 6·68 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}2$–$0{\cdot}3$

Mean width, 46·675 μ
Standard deviation, 3·74 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}01$

Table 6. *Bunostomum trigonocephalum*

Length (μ)	Observed frequency	Calculated frequency	Width (μ)	Observed frequency	Calculated frequency
77·5	0	0·52	35	2	0·031
80	3	1·36	37·5	0	0·646
82·5	3	3·71	40	5	5·14
85	12	7·98	42·5	25	19·0
87·5	20	13·52	45	43	33·89
90	27	17·74	47·5	19	27·94
92·5	11	19·23	50	6	11·13
95	7	16·17	52·5	0	2·0
97·5	9	10·76			
100	7	5·57			
102·5	1	2·4			
105	0	0·78			
107·5	0	0·21			

Mean length, 91·9 μ
Standard deviation, 5·131 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}1$

Mean width, 45·64 μ
Standard deviation, 2·79 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}5$

Table 7. *Chabertia ovina*

Length (μ)	Observed frequency	Calculated frequency	Width (μ)	Observed frequency	Calculated frequency
75	0	0·07	42·5	0	0·13
77·5	1	0·3	45	2	2·3
80	1	1·3	47·5	13	15·0
82·5	4	4·3	50	42	36·6
85	10	10·2	52·5	30	33·5
87·5	16	17·6	55	12	11·1
90	25	22·2	57·5	1	1·4
92·5	16	20·5	60	0	0·07
95	17	13·7			
97·5	9	6·7			
100	1	2·4			
102·5	0	0·75			

Mean length, 90·60 μ
Standard deviation, 4·388 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}3$–$0{\cdot}5$

Mean width, 51·00 μ
Standard deviation, 2·397 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}5$–$0{\cdot}7$

Table 8. *Ostertagia* spp.

Length (μ)	Observed frequency	Calculated frequency	Width (μ)	Observed frequency	Calculated frequency
80	0	0·64	40	0	1·07
82·5	1	2·04	42·5	5	4·41
85	2	5·7	45	13	12·66
87·5	22	12·23	47·5	25	23·54
90	20	19·13	50	29	27·11
92·5	19	21·67	52·5	13	19·31
95	16	18·82	55	12	8·89
97·5	9	11·84	57·5	3	2·52
100	8	5·43	60	0	0·49
102·5	2	1·91			
105	1	0·48			
107·5	0	0·11			

Mean length, 92·425 μ
Standard deviation, 4·49 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}1$–$0{\cdot}2$

Mean width, 49·502 μ
Standard deviation, 3·588 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}5$

Table 9. *Oesophagostomum venulosum*

Length (μ)	Observed frequency	Calculated frequency	Width (μ)	Observed frequency	Calculated frequency
82·5	0	0·39	42·5	0	0·51
85	1	0·74	45	6	3·95
87·5	1	1·74	47·5	10	16·15
90	0	3·56	50	37	31·39
92·5	7	6·28	52·5	33	30·39
95	14	9·65	55	10	14·03
97·5	15	13·21	57·5	3	3·22
100	19	14·83	60	1	0·35
102·5	13	14·79	62·5	0	0·02
105	11	12·77			
107·5	8	9·55			
110	1	6·21			
112·5	4	3·56			
115	3	1·67			
117·5	2	0·70			
120	1	0·23			
122·5	0	0·11			

Mean length, 101·2 μ
Standard deviation, 6·552 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}1$

Mean width, 51·10 μ
Standard deviation, 2·858 μ
Goodness of fit, $P_{\chi^2} = 0{\cdot}3$–$0{\cdot}5$

V. EGG DIFFERENTIATION

From the data cited in the foregoing sections a method of egg differentiation has been devised. This method is based upon the statistical chances of eggs of different species falling into particular size classes. To some extent the 'free areas' methods of Tetley (1941*a*, 1949) have been incorporated, but basically the principle is different from Tetley's in so far as no attempt is made to identify individual eggs. The differentiation is made by reference to the gross pattern produced by overlapping populations of size groups which differ numerically according to the size of each species population in the sample.

In the ensuing section the theoretical analysis is developed and method of interpretation of results is described.

(*a*) *Theoretical distribution of the linear measurements of eggs*

Table 10 shows the calculated distribution (except in the case of *T. vitrinus*) of percentages of the eggs of the different species within various limits of length and width. Five classes are indicated by the letters A, M, J, F and Q, so that

class A includes eggs of length 60–77·5 μ,
class M includes eggs of length 102·5–125 μ,
class J includes eggs of width 27·5–37·5 μ,
class F includes eggs of width 40–50 μ,
class Q includes eggs of length 52·5–62·5 μ.

Each of these classes includes egg dimensions of a fraction of some or all of the species under consideration, and each class can be expected to contain percentages of all the eggs of species as shown below:

Class A: 87·30 % of all *H. contortus* eggs,
63·31 % of all *C. curticei* eggs,
8·78 % of all *T. axei* eggs,
0·52 % of all *B. trigonocephalum* eggs,
0·35 % of all *C. ovina* eggs.

Class M: 0·35 % of all *T. axei* eggs,
49·19 % of all *T. vitrinus* eggs,
3·44 % of all *B. trigonocephalum* eggs,
0·75 % of all *C. ovina* eggs,
2·5 % of all *Ostertagia* spp. eggs,
49·63 % of all *O. venulosum* eggs.

Class J: 0·6 % of all *H. contortus* eggs,
95·99 % of all *C. curticei* eggs,
40·9 % of all *T. axei* eggs,
1·0 % of all *T. vitrinus* eggs,
0·68 % of all *B. trigonocephalum* eggs.

Class F: 99·3 % of all *H. contortus* eggs,
4·01 % of all *C. curticei* eggs,
59·11 % of all *T. axei* eggs,
97·10 % of all *B. trigonocephalum* eggs,
53·98 % of all *C. ovina* eggs,
89·0 % of all *T. vitrinus* eggs,
68·79 % of all *Ostertagia* spp. eggs,
52·0 % of all *O. venulosum* eggs.

Class Q: 0·07 % of all *H. contortus* eggs,
10·0 % of all *T. vitrinus* eggs,
2·22 % of all *B. trigonocephalum* eggs,
46·02 % of all *C. ovina* eggs,
31·21 % of all *Ostertagia* spp. eggs,
48·01 % of all *O. venulosum* eggs.

From these five classes, since the distribution of length and width is considered to be bivariate, one more class can be derived:

Class F (of A) which represents the following percentages of eggs which fall into class A and into class F—
99·3 % of 87·3 % or 86·9 % of all *H. contortus* eggs,
63·31 % of 4·01 % or 2·54 % of all *C. curticei* eggs,
8·78 % of 59·11 % or 5·19 % of all *T. axei* eggs,
0·52 % of 97·10 % or 0·50 % of all *B. trigonocephalum* eggs,
0·35 % of 53·98 % or 0·19 % of all *C. ovina* eggs.

(b) *Interpretation of class distributions within a mixed sample of eggs*

If, in a mixed sample, the number of eggs which fall into class A is a, into class F is f, into class M is m, into class Q is q, into class J is j, into class F (of A) is f (of a), then the total number of eggs (N) in the sample is $(j+f+q)$, and the following interpretations of the class distributions can be made. In each case the contributions of species other than those with a large percentage of their eggs in the class under consideration have been ignored. The significance of the ignored contributions will be discussed at a later stage when an estimate of their sizes can be made.

Interpretation of class F (of A)

The species with a large percentage of its eggs in this class is *H. contortus*. If its eggs are present in the mixed population 86·69 % will be in class F (of A) and an estimate of the total population of *H. contortus eggs* (P_1) will be

$$P_1 = \tfrac{1}{0}\,(f \text{ (of } a)).$$

Interpretation of classes A and F (of A)

The composition of these two classes differs mainly in relation to *C. curticei* eggs.
Thus $(a-f$ (of $a))$ represents:

0·61 % of all *H. contortus* eggs,
61·07 % of all *C. curticei* eggs,
2·59 % of all *T. axei* eggs,
0·02 % of all *B. trigonocephalum* eggs,
0·16 % of all *C. ovina* eggs,

and if eggs of *C. curticei* are present 61·07 % of them will be included in the total $(a-f$ (of $a))$, thus an estimate of the total population of *C. curticei* eggs (P_2) will be

$$P_2 = \tfrac{1}{0}\,(a-f \text{ (of } a)).$$

Interpretation of class J

Two species, *C. curticei* and *T. axei*, have large percentages of their eggs in this class. If eggs of these species are present 95·99 % of *C. curticei* eggs (P_2) and 40·9 % of *T. axei* eggs (P_3) will be in class J and an estimate of the total population of *T. axei* eggs will be

$$P_3 = \tfrac{1}{4}\,(j - \tfrac{9}{1}\tfrac{6}{0}\,(P_2)).$$

Interpretation of class M

Two species, *T. vitrinus* and *O. venulosum*, have significant percentages of their eggs in this class, and if their eggs are present 49·19 % *T. vitrinus* eggs and 49·63 % *O. venulosum* eggs will be in class M. Thus an estimate of the total populations of *T. vitrinus* eggs (P_4) plus *O. venulosum* eggs (P_5) will be

$$P_4 + P_5 = 2m.$$

From these interpretations it is possible to arrive at relationships between the totals of eggs falling into various classes and the specific populations of a mixed sample.

Table 10. *The calculated percentage frequency distribution of length and width of the eggs of the species under consideration*

Length

	A								M																
	60	62·5	65	67·5	70	72·5	75	77·5	80	82·5	85	87·5	90	92·5	95	97·5	100	102·5	105	107·5	110	112·5	115	117·5	120	122·5	125
P_1	0·44	1·48	3·38	9·81	16·89	20·98	20·24	14·08	7·61	2·96	0·89	0·21	0·03	—	—	—	—	—	—	—	—	—	—	—	—	—	—
P_2	—	—	0·19	1·03	4·26	11·37	20·98	25·48	20·34	11·20	3·99	0·99	0·17	—	—	—	—	—	—	—	—	—	—	—	—	—	—
P_3	—	—	—	—	—	0·96	3·05	4·77	9·07	13·65	17·51	17·90	14·6	10·15	5·59	2·5	0·89	0·28	0·06	0·01	—	—	—	—	—	—	—
P_4	—	—	—	—	—	—	—	—	—	0·47	0·82	1·93	3·72	6·41	9·92	12·67	14·86	14·37	12·77	9·35	6·28	3·49	1·78	0·75	0·28	0·08	0·04
P_5	—	—	—	—	—	—	—	0·52	1·36	3·715	7·98	13·52	17·74	19·23	16·17	10·76	5·57	2·40	0·78	0·21	0·05	—	—	—	—	—	—
P_6	—	—	—	—	—	—	0·07	0·28	1·31	4·28	10·17	17·61	22·24	20·46	13·73	6·71	2·39	0·75	—	—	—	—	—	—	—	—	—
P_7	—	—	—	—	—	—	—	—	0·64	2·04	5·70	12·23	19·13	21·67	18·82	11·84	5·43	1·91	0·48	0·11	—	—	—	—	—	—	—
P_8	—	—	—	—	—	—	—	—	—	0·39	0·74	1·74	3·56	6·28	9·65	13·21	14·83	14·79	12·77	9·55	6·21	3·56	1·67	0·70	0·23	0·11	—

Width

	J										Q				
	25	27·5	30	32·5	35	37·5	40	42·5	45	47·5	50	52·5	55	57·5	60	62·5
P_1	—	—	—	—	—	0·6	8·91	31·39	40·96	16·17	1·90	0·07	—	—	—	—
P_2	—	0·01	0·77	14·37	48·91	31·93	3·93	0·08	—	—	—	—	—	—	—	—
P_3	—	0·07	0·59	3·52	12·17	24·55	28·95	19·95	7·98	1·93	0·3	—	—	—	—	—
P_4	—	—	—	—	—	1	9	6	36	15	23	7	3	—	—	—
P_5	—	—	—	—	0·03	0·65	5·14	19·0	33·89	27·94	11·13	2·03	0·19	—	—	—
P_6	—	—	—	—	—	—	—	0·13	2·25	14·98	36·62	33·51	11·08	1·36	0·07	—
P_7	—	—	—	—	—	—	1·07	4·41	12·66	23·54	27·11	19·31	8·89	2·52	0·49	—
P_8	—	—	—	—	—	—	—	0·51	3·95	16·15	31·39	30·39	14·03	3·22	0·35	0·02

P_1 refers to *H. contortus* — P_5 refers to *B. trigonocephalum*
P_2 refers to *C. curticei* — P_6 refers to *C. ovina*
P_3 refers to *T. axei* — P_7 refers to *Ostertagia* spp.
P_4 refers to *T. vitrinus* — P_8 refers to *O. venulosum*

Table 11

Sample...	I		II		III		IV		V		VI		VII		VIII		IX		X		XI		XII		XIII		XIV		XV	
	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.	A.	E.
H. contortus (P_1)	21	22	34	31	13	11	36	39	11	13	44	47	26	29	7	13	0	0	0	1	0	3	0	2	0	1	0	1	0	1
C. curticei (P_2)	7	5	10	11	11	12	4	5	10	10	3	4	7	7	10	10	0	0	2	2	10	11	0	0	0	0	0	0	0	0
T. axei (P_3)	17	20	7	0	13	5	2	0	29	21	16	2	18	14	23	11	18	13	0	0	20	8	10	5	0	0	3	0	24	10
T. vitrinus (P_4) *O. venulosum* (P_8)	12	16	28	22	8	20	21	26	10	12	4	4	19	18	2	10	9	22	23	16	20	26	10	16	32	40	28	28	4	1+
B. trigonocephalum (P_5) *C. ovina* (P_6) *Ostertagia* spp. (P_7)	43	37	21	36	55	52	37	30	40	44	33	36	30	32	58	56	73	65	75	81	50	52	80	77	68	59	69	71	72	75

A. = Actual; E. = Estimate.

The relationships are stated below for a mixed sample of size N:

$$H.\ contortus\ (P_1) = \tfrac{1}{5}0(f\ (\text{of } a)),$$
$$C.\ curticei\ (P_2) = \tfrac{1}{5}0(a - f\ (\text{of } a)),$$
$$T.\ axei\ (P_3) = \tfrac{1}{4}0(j - \tfrac{0}{1}\tfrac{6}{0}\tfrac{6}{0}(P_2)),$$
$$T.\ vitrinus\ (P_4) + O.\ venulosum\ (P_5) = 2m,$$
$$B.\ trigonocephalum\ (P_6) + C.\ ovina\ (P_6) + Ostertagia\ \text{spp.}\ (P_7) = N - \Sigma P_1P_2P_3P_4P_5.$$

(c) *Method of application of the derived relationships to differential egg counts*

The proportion of eggs of different species in a mixed sample can be estimated by using the relationships derived above.

The method involves classifying 100 eggs from sheep faeces into the classes previously defined in this paper and tabulating the totals of eggs which fall into each class.

Faeces are prepared for sampling as in a normal egg count (McMaster slide) method, and are viewed through a microscope fitted with a camera lucida and equipped and calibrated as previously described in this paper. Linear measurements are not made but the images of the eggs are superimposed upon that of a scale (described later) which is constructed so that direct classification of the eggs is possible. The totals for each class are recorded in tabular form under their respective class headings. *Nematodirus* and *Strongyloides* eggs are recorded separately and do not contribute to the total of 100 eggs.

Substitution of the class totals in the derived relationships gives estimates of the various specific populations.

Table 11 shows the results obtained when the method was used with mixed populations of eggs. These mixtures were prepared by random sampling of eggs which had been classified after dissection from identified worms.

VI. THE CLASSIFYING SCALE AND ITS CONSTRUCTION

Fig. 6 shows the classifying scale. The horizontal and vertical lines, taken in conjunction with the main axes, represent the linear limits of the various classes and direct classification of each egg is thus possible. (N.B. Eggs which fall into classes A, F, and F (of A) are recorded in each respective class into which they fall, i.e. each such egg is recorded three times.) The rectangle S represents the maximum dimensions of *Strongyloides* and the upper limits of Q and M represent the minimum dimensions of *Nematodirus* (N).

The scale is prepared by making a large proportional drawing and photographing it so that the required reduction in size is obtained. Use of a standard line, which must be of a certain length at the reduced size, is useful to obtain the correct reduction and to ensure that paper shrinkage has not taken place during the preparation of the photographic positive which forms the scale.

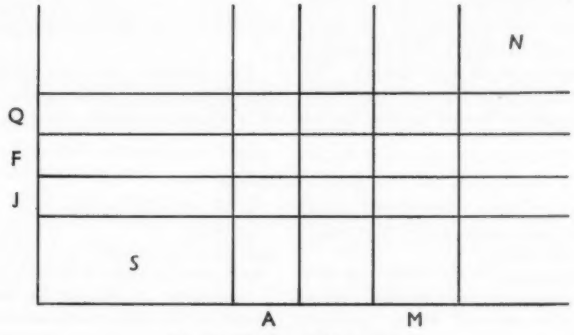

Fig. 6. The classifying scale
($S = Strongyloides$, $N = Nematodirus$)

VII. DISCUSSION OF METHOD OF EGG DIFFERENTIATION

The method described for differentiation of eggs in sheep faeces could be generally applied where only the same species are encountered. A new series of equations would be necessary where *Gaigeria pachyscelis*, *O. columbianum* and *Ostertagia marshalli* occur. The limitations of the method are concerned mainly with the number of eggs to be classified. The minimum number which can be usefully employed is one hundred, but better estimates might be expected if large numbers were used. The use of large numbers presents no difficulty where the proportion of different species occurring in a flock is required, because the individual readings

can be pooled. Where estimates for individual sheep are to be made the method is more laborious. For flock examinations the time required for classification and counting is approximately twice that necessary for counting by the ordinary McMaster method.

The advantages of egg differentiation are:

(1) The method is more rapid than culture and subsequent identification of larvae.

(2) There is less variation than in larval culture methods in which the conditions in the culture affect the migration and recovery of species differentially.

(3) An operator skilled in larval identification is not required.

The disadvantages are

(1) The method can only be applied to fresh faeces, and material cannot be stored for future reference and checking.

(2) The accuracy of differentiation increases with number of eggs classified, but the results can only be regarded as an estimate which is more accurate for the more numerous species in any sample.

This latter point also applies to larval identification, but whereas a positive result in the larval identification method always has some meaning, a small positive result in the egg differentiation method may not.

VIII. SUMMARY

1. The width and length of eggs of *Haemonchus contortus*, *Cooperia curticei*, *Trichostrongylus axei*, *T. vitrinus*, *Bunostomum trigonocephalum*, *Chabertia ovina*, *Oesophagostomum venulosum* and *Ostertagia* spp. were measured.

2. Frequency distributions of the linear dimensions of the eggs of each species are given, together with the frequencies calculated on the assumption that the distributions are normal.

3. A method of species differentiation based on egg measurements is described, the method being based upon the statistical chances of eggs of different species falling into particular size classes.

4. The application of the method and the construction of a classifying scale are described.

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A NEW MICROSPORIDIAN, *NOSEMA LOCUSTAE* N.SP., FROM THE FAT BODY OF THE AFRICAN MIGRATORY LOCUST, *LOCUSTA MIGRATORIA MIGRATORIOIDES* R. & F.

By ELIZABETH U. CANNING

Department of Parasitology, London School of Hygiene and Tropical Medicine

(With 20 Figures in the Text)

INTRODUCTION

On 7 March 1953 I received several specimens of *Locusta migratoria* from Mr T. Singh, who found them dying in the breeding cages at the Imperial College, London. Microscopic examination showed that the fat body contained large numbers of oval-shaped spores belonging to a microsporidian.

At present there are only five records of microsporidia from the Orthoptera. Wieser (1947) records *Nosema periplanetae* Lutz & Splendore 1903 from *Periplaneta americana* (L) and *Plistophora kudoi*, Sprague & Ramsay 1942 from *Blatta orientalis* (L). In addition, Kudo (1924) has recorded microsporidia from *Gryllus campestris* (L) and *Platycleis grisea* (Fabr.), but the genera and species are uncertain. The only record of a microsporidian from the Acrididae is by Steinhaus (1951), who found 'a microsporidian that is probably a new species of *Nosema*' in the fat body of *Melanoplus bivittatus* (Say), *M. mexicanus* (Sauss), and *M. dawsoni* (Scudder).

MATERIALS AND METHODS

Infected locusts were obtained from the Imperial College Field Station, Silwood Park. Healthy locusts for experimental infections were obtained from the Anti-Locust Research Centre, British Museum (Natural History), London.

In all cases examinations were first made on fresh material in 0·75% sodium chloride solution. Permanent preparations in the form of smears were stained with Giemsa's solution or Ehrlich's haematoxylin and eosin. Material for sectioning was fixed in Carnoy solution and stained by the Giemsa-colophonium method or with Ehrlich's haematoxylin and eosin. The best results were obtained with the Giemsa techniques. Spores were treated with nitric acid (Thelohan, 1894) to show the polar capsule. Extrusion of the polar filament was effected easily by mechanical pressure, and Fontana staining gave good results in staining the extruded filament.

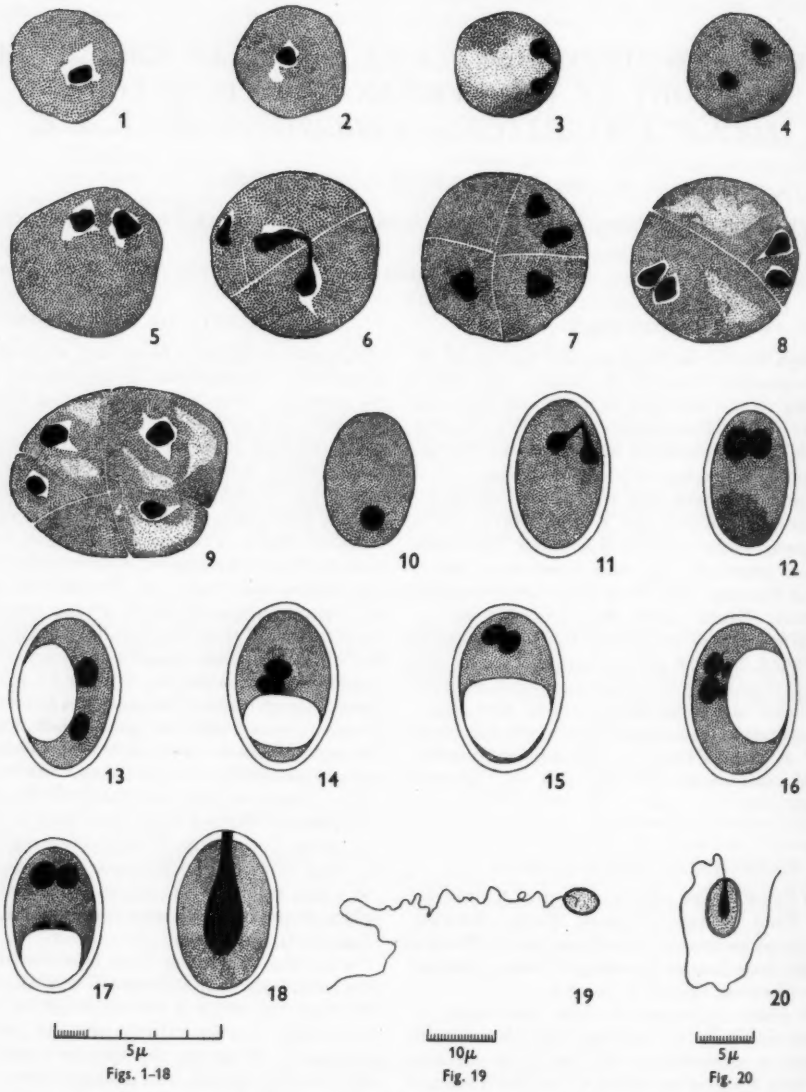
DESCRIPTION OF THE PARASITE

Sections of the fat body in an early stage of infection showed the intracellular developmental stages of the parasite. Planont stages passing through the gut wall have not yet been observed.

The earliest schizont is a small spherical body with a single central or slightly eccentric nucleus, measuring 2·5–3·5 μ diameter. The cytoplasm stains pink or blue-pink, and the nucleus a deep red with Giemsa's stain. The nucleus appears to be surrounded by a clear area but otherwise the cytoplasm is homogeneous (Figs. 1, 2). The schizont grows and undergoes nuclear division, giving rise first to a binucleate and then to a quadrinucleate body. The binucleate schizonts measure from 3 to 5 μ and the quadrinucleate schizonts from 5 to 6·6 μ in diameter. The cytoplasm becomes less homogeneous as division occurs, and the quadrinucleate schizonts frequently show irregularly occurring lightly stained patches in the cytoplasm. Nuclear division of the young schizont was observed (Fig. 3) but no cytoplasmic furrows were developed at this stage. Divisions do not appear to proceed regularly, and in many cases two nuclei occur together, separated from the others by a cytoplasmic furrow, while other areas of cytoplasm are devoid of nuclei (Figs. 7–9).

Schizonts with more than four nuclei were not observed. Asexual reproduction continues inside the host cell until it becomes full of the round uninucleate bodies which are the products of schizogony. These are the sporonts, each of which will develop directly into a single spore.

The sporont becomes oval (Fig. 10) and then develops a thick wall. The nucleus now divides (Fig. 11), giving rise to two large nuclei situated towards one end of the spore. There is often a large vacuole at the other end, but this may occur laterally (Figs. 13, 16). The nuclei divide, budding off two very small chromatin bodies which come to lie at the edge of the vacuole (Figs. 16, 17). These are possibly the nuclei of the polar capsule. Development of the polar capsule was not observed



Figs. 1–20. All drawings are made from smear or section preparations, stained with Giemsa's solution. Figs. 1, 2. Uninucleate schizonts. Fig. 3. Schizont undergoing nuclear division. Figs. 4, 5. Binucleate schizonts. Fig. 6. Irregular division of schizont. Figs. 7–9. Quadrinucleate schizonts. Fig. 10. Sporont. Fig. 11. Immature spore with dividing nucleus. Fig. 12. Spore with two nuclei. Figs. 13–15. Spores showing vacuole and two large nuclei. Figs. 16, 17. Mature spores. Fig. 18. Spore treated with nitric acid showing the polar capsule. Fig. 19. Spore stained by Fontana's method showing extruded filament. Fig. 20. Spore stained by Fontana's method showing filament and polar capsule.

but, on treatment of fresh spores with 66 % nitric acid heated to 36° C., the polar capsule becomes visible as a flask-shaped body occupying two-thirds of the length of the spore and one-half the width. The neck of the flask opens at one end of the spore (Fig. 18). The polar filament is easily extruded by mechanical pressure, and measurements varied between 75 and 156 μ, the variation probably being due to the degree of extrusion (Figs. 19, 20). The mature spore measures 3·5–5·5 μ in length and 1·5–3·5 μ in width. The spore wall is very thick and refractile, rendering it impossible to see any internal structure of the spore in the fresh state.

THE EFFECT OF THE PARASITE ON THE HOST

The parasites enter the cells of the fat body of the host where they undergo schizogony. After a while the cell walls begin to break down, the cell outlines soon becoming invisible so that the host nuclei appear to lie scattered amongst the large masses of schizonts. Sooner or later the host nuclei degenerate and finally, when sporogony begins, there is little left of the host tissue, and the fat body becomes a mass of spores.

In healthy insects the fat body is a translucent bright yellow structure forming a complete sheath round the intestine and gonads, and in the female it also occurs between the ovaries. It is not an extensive body, especially in the older insects, but remains as a thin layer. The first macroscopic change observed is that the fat body begins to lose its translucence and becomes opaque and creamy in colour. At this stage microscopically the fat cells are full of the opaque spores which are present in such large numbers as to impart the creamy colour to the fat tissue. The fat itself having been used up by the parasite, the translucence is lost. As the infection proceeds, the creamy mass enlarges through the production of large numbers of spores so that eventually all the spaces in the haemocoel are filled. At this stage little or no host tissue is left, and the haemocoel appears to be full of spores. Later pink patches begin to occur in the creamy body and finally the whole mass takes on a deep rose colour throughout. The abdomen, and even the thorax, of an individual at this stage is a deep red colour owing to the parasitic mass showing through the chitin. The red pigment does not appear to be in the spores themselves but rather surrounding the spores. The nature of the pigment is not yet established.

The parasites invade the cells of the fat body almost exclusively, the reproductive organs, accessory glands, malpighian tubules, etc., being left untouched.

EXPERIMENTAL INFECTIONS

Several attempts were made to produce the infection experimentally but only light infections were produced. Assuming that the spores are introduced *per os*, as in other microsporidia, the following two methods were employed.

Thirty newly emerged adults were placed in a breeding cage and were fed daily with grass over which some of the fat tissue from a heavily infected locust was smeared. The locusts were obtained from the Anti-Locust Research Centre, and several were examined to ensure they were free from infection before the experiment.

They were first fed infective food on 10 March 1953. One of the locusts appeared diseased on 16 March and was dead on 17 March. Examination showed that spores were present, but not in sufficiently large numbers to have caused the death of the host, as locusts with a natural infection remained alive with the fat body almost entirely replaced. On 20 March another locust was dead but again only a few spores were present. On 24 March two more died and one of these showed a heavier infection though not nearly to the extent of the natural infection.

After one month with the infections getting no heavier the second method was employed. As the locusts preferred to resort to cannibalism rather than eat unsuitable food, it is possible that they avoided eating blades of grass with the lumps of fat tissue on them. To ensure even distribution of the spores on the grass the fat tissue was mixed with distilled water and the mixture poured on and thoroughly mixed with the grass. The grass was then partially dried to avoid a high humidity in the cages, and thereby avoid deaths from this cause, and only sufficient grass was fed to the locusts to ensure all was eaten. As before, this treatment was carried out daily and again no heavy infections were produced.

MORTALITY OF INFECTED LOCUSTS

Owing to the failure with experimental infections I am unable to produce any first-hand information on the mortality of the infected locusts, but the following information was kindly supplied by Mr T. Singh (Imperial College) and Mr W. Ives (Imperial College Field Station).

On 9 February Mr Singh received sixty fifth-stage hoppers from the Imperial College Field Station. On emergence to adult stage the locusts were separated into pairs, male and female, and were transferred to 3 lb. glass jars containing moist sand at the bottom. The sand was moistened with 10 c.c. water to 100 c.c. sand. The relative humidity was 64 % and the jars were kept in a constant temperature room where the temperature was 25° C. at

night rising to 28–30° C. during the day. Fresh grass was fed to the locusts daily. By 19 February fifteen out of the sixty locusts had died. The remaining locusts were discarded and a fresh supply brought from the field station.

The second supply of twenty-three fifth-stage hoppers were received on 26 February. On emergence the pairs were this time transferred to 7 lb. glass jars with gravel at the bottom and sand tubes sunk into the gravel to bring the sand level to that of the gravel. The R.H. and temperature were the same as before. The first locusts died on 4 March, and by 6 March six were dead. These were the specimens given to me for examination.

The third supply were kept in larger breeding cages under the same condition of R.H. and temperature. No deaths were recorded and examination showed them to be free of infection.

The majority of the locusts at the Imperial College Field Station were heavily infected. However, some of the cages contained locusts completely free of infection and it is probable that Mr Singh's third supply of locusts came from these cages. Though, at the time, no serious mortality rate was recorded, previously as many as fifteen locusts died per day in a cage containing about 100–150 locusts. The red appearance of the abdomen had been observed in these locusts which implies that these deaths were due to the microsporidian.

From the above information, though far from complete, it would appear that the microsporidian is pathogenic to its host. Nevertheless, from the Imperial College Field Station records it seems that it is not always pathogenic and it is probable therefore that the disease may remain latent until external conditions become unfavourable—such as too high a humidity, overcrowding—and then the disease takes its course.

INFECTIONS OF OTHER SPECIES OF LOCUST

Examinations of *Schistocerca gregaria* Forsk, from the Anti-Locust Research Centre and the Imperial College Field Station revealed no infections, but one specimen sent recently from the Colonial Insecticides Research Unit, Porton, was fairly heavily infected. The spore measurements range from 4·5 to 5·5 μ long by 2·5 to 3·5 μ broad. It was impossible to extrude the polar filament by mechanical pressure as was easily done in the case of infections in *Locusta migratoria*. I cannot say at this stage if it is the same species or not.

DISCUSSION

Since the sporont of the microsporidian gives rise to a single spore it qualifies for inclusion in the genus *Nosema*. Measurements of the spore, filament and vegetative phases do not fall within the ranges of measurements for previously described species. For this reason I consider it to be a distinct species and propose the name *Nosema locustae* n.sp. Further work on the biology and pathogenicity of the parasite is in progress, but in view of the possible economic importance in the control of the locust this preliminary report is submitted for publication.

I should like to express my thanks in particular to Professor P. C. C. Garnham of the London School of Hygiene and Tropical Medicine for advice and assistance in the preparation of this paper, and to Mr T. Singh and Mr W. Ives for placing at my disposal the information on the mortality of the locusts.

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PARASITIC NEMATODA COLLECTED BY THE AUSTRALIAN NATIONAL ANTARCTIC RESEARCH EXPEDITION: HEARD ISLAND AND MACQUARIE ISLAND, 1948–1951

By PATRICIA M. MAWSON, M.Sc.

Zoology Department, University of Adelaide

(With 19 Figures in the Text)

INTRODUCTION

The nematodes described here were collected by biologists of the Australian National Antarctic Research Expedition based at the subantarctic stations at Macquarie Island and Heard Island, over the years 1948–51. The biologists concerned were:

Year	Heard Island	Macquarie Island
1948	A. Gilchrist	R. Kenny
1949	R. G. Chittleborough	N. M. Haysom
	E. H. M. Ealey	T. Manefield
1950	L. Gibbney	E. Shipp
	P. Young	W. Taylor
1951	K. Brown	J. Bunt
	M. Downes	E. Lindholm

I am most grateful to the A.N.A.R.E. for the opportunity to examine the material and to their biologists for the well-preserved and well-labelled collections. The specimens from Heard Island 1949 are the most numerous and are from the widest range of hosts and of locations in the body, and it is among these that new species have been found.

Collection ciphers for both parasite and host are given where available. In, for example, the cipher MZ/49/P45 (B27), M stands for Macquarie Island (H used for Heard Island), Z stands for zoology, 49 indicates the year, 1949; P45 is the number of the specimen; (B27) is the number of the host. Where several collections of the same species of parasite have been taken from the same host species at the same place and year the first two symbols are given only for the first specimen of the series.

LIST OF PARASITES EXAMINED ARRANGED UNDER THEIR HOSTS

Parasites of mammals

Hydrurga leptonyx (Blainville). *Contracaecum osculatum* (Rud.), *C. radiatum* (Linst.), *Parafilaroides hydrurgae* n.sp., Heard Island; *Stomachus similis* (Baird), Heard Island and Macquarie Island; *Terranova piscium* (Rud.), Macquarie Island.

Mirounga leonina Linn. *Stomachus similis* (Baird), *Contracaecum osculatum* (Rud.), Heard Island and Macquarie Island; *Contracaecum radiatum* (Linst.), *Filaria* (s.l.) sp., Heard Island.

Arctocephalus forsteri (Lesson). *Contracaecum osculatum* (Rud.). Macquarie Island.

Parasites of birds

Diomedea melanophris Temm. *Stomachus* sp., Macquarie Island.

Phoebetria palpebrata (Forster). *Seuratia shipleyi* (Stoss.), *Paranisakiopsis* sp., Heard Island.

Macronectes gigantea (Gmelin). *Capillaria convoluta* (Fourment), Heard Island.

Oceanites oceanicus (Kuhl). *Stegophorus heardi* n.sp., Heard Island.

Pelecanoides georgicus Murphy & Harper. *Stegophora heardi* n.sp., Heard Island.

Pygoscelis papua Forster. *Stegophora paradeliae* (Johnston & Mawson), *Contracaecum heardi* n.sp., Heard Island; *Stomachus* sp., Macquarie Island.

Eudyptes cristatus (Miller). *Stegophora paradeliae* (Johnston & Mawson), *Contracaecum* sp., Heard Island.

Eudyptes chrysolophus Brandt. *Stegophora paradeliae* (Johnston & Mawson), *Contracaecum heardi* n.sp., Heard Island.

Aptenodytes patagonica Miller. *Contracaecum* sp., Heard Island.

Phalacrocorax atriceps nivalis Falla. *Desmidocercella australis* n.sp., Heard Island.

Eudyptes schlegeli Tinsch. *Terranova* sp., *Stomachus* sp., Macquarie Island.

Notothenia coriiceps Richardson. *Ascarophis nototheniae* (Johnston & Mawson), *Contracaecum* sp., Heard Island.

Notothenia cyanobrancha Richardson. *Ascarophis nototheniae* (Johnston & Mawson), *Contracaecum* sp., Heard Island.

Notothenia macrocephala Günther. *Stomachus* sp., larva, Macquarie Island.

'Fish' (1) 1/49, *Stomachus* sp., Macquarie Island.
(2) no data, *Stomachus* sp., Macquarie Island.

Muraenolepsis sp. *Stomachus* sp., Macquarie Island.

SPECIMENS NOT IDENTIFIED OWING TO POOR PRESERVATION

Hosts

Mirounga leonina Linn. MZ/49/P30 (M25).
Diomedea melanophris Temm. HZ/49/244 (50).
Pygoscelis papua Forster. MZ/49/P50 (B30), MZ/49/P32.
Eudyptes chrysolophus Brandt. HZ/49/640 (631).
Phalacrocorax atriceps nivalis Falla. HZ/49/694 (693).

DESCRIPTIONS OF PARASITES

Stomachus similis (Baird)

This species is more common in animals from Macquarie Island than from Heard Island; among nematode-infested hosts, it was taken from three of ten specimens of *Mirounga leonina* and from one of four *Hydrurga leptonyx* at Heard Island, and from four of seven *Mirounga leonina* and four of nine *Hydrurga leptonyx* at Macquarie Island. The collections in which the species is present are:

Hydrurga leptonyx: HZ/49/806 (803); MZ/48/Y2 (SL2), Y8 (SL8); MZ/49/P13 (M6), P28 (M12); MZ/51/Y1.
Mirounga leonina: HZ/49/165 (163), /237 (235), /316 and 317 (314); MZ/48/Y15 (M15); and MZ/49/P4 (M2), /P12 (M5), /P29 (M20).

Stomachus spp.

Immature worms belonging to the genus *Stomachus* were taken from several hosts and may be placed in three groups:

(1) Those from the black-browed Mollymawk, *Diomedea melanophris*, MZ/49/P45 (B27). This collection comprises young forms found free in the host stomach. They are without larval tooth, but with labia only slightly developed. The lengths of the ventriculus and oesophagus are in the same proportions to one another as in *S. diomediae*; it is probable that they belong to this species.

(2) Collections from various fish, as follows: *Notothenia macrocephala*, MZ/49/P30; *Muraenolepis* sp., MZ/51/Y4; and 'fish', Macquarie Island. These collections resemble the larvae of *Stomachus* sp. described by Johnston & Mawson (1945), from various fish, and considered then as likely to be young forms of *Stomachus similis* or *S. simplex*, more probably the former.

(3) Two collections from *Pygoscelis papua*, MZ/49/P40, and MZ/51/Y3. These larvae, few in number, are large worms, up to 25 mm. long, 0·55 mm. diameter, and possess a larval tooth. They differ from group (2) above in two main points; first, they occur free in the host stomach, not, as in the specimens from fish, encapsulated in the body cavity; and, secondly, the oesophagus, instead of being of more or less even width throughout, is distinctly wider in its posterior two-fifths. It is probable that the adult of this species has not yet been recorded.

Terranova piscium (Rud.)

This species was not present in any collection from Heard Island; at Macquarie Island it was taken from seven of nine nematode-infested sea leopards, *Hydrurga leptonyx*, MZ/48/Y1 (SL1), /Y2 (SL2), /Y3 (SL3), /Y4 (SL4); and MZ/49/P1 (M1), /P13 (M6), /P21 (M8). An immature specimen was taken also from *Eudyptes schlegeli*, MZ/49/P24.

Contracaecum radiatum (Linst.)

This species was present only in collections from Heard Island, from *Hydrurga leptonyx*, HZ/51/P13 (SL1), and *Mirounga leonina*, HZ/49/357 and 359 (356), /482 (480); and HZ/50/P5.

Contracaecum osculatum (Rud.)

This species appears to be the most cosmopolitan and most abundant stomach parasite of seals. It was present in the following collections:

Hydrurga leptonyx: HZ/49/129 and 130 (127), /141 (140); and HZ/50/P9, /P22 (S10).
Mirounga leonina: HZ/49/213, 214, 215, 216 and 217 (207), /237 (235), /305 (303), /358 (356), /469 (468), /501 (500), /164 (163); HZ/50/P5; MZ/48/Y22 (SE22); and MZ/49/P6 (M4), /P43 (M34).
Arctocephalus patagonica: MZ/50/P43 (M34).

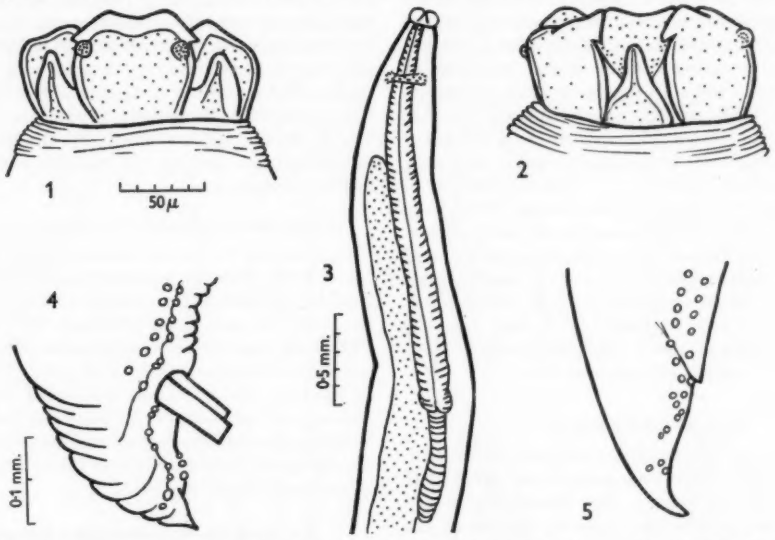
Contracaecum heardi n.sp. (Fig. 1–5)

A new species of the genus *Contracaecum* was taken from various penguins at Heard Island, namely: the gentoo, *Pygoscelis papua* (type host), HZ/49/204 (145), /107 (106*a*), /175 (173), 312 (311); the rock-hopper, *Eudyptes cristatus*, HZ/49/448 (446); the crested penguin, *Eudyptes chrysolophus*, HZ/49/629 (620); and *Aptenodytes patagonica*, HZ/49/183 (182). The males are up to 23 mm. long, the females to 48 mm. The lips are about as long as wide, with small antero-lateral projections, and with very narrow lateral flanges. A folded cuticular collar is present but is not outstanding. The interlabia are wide at the base, but narrow suddenly, ending as a digitiform projection. The oesophagus is of almost the same width throughout, and ends in a small ventriculus. The proportions of the parts of the alimentary tract at various stages of the life cycle

are given in Table 1. The vulva lies at 1/4·6–1/5 of the body length from the anterior end of the worm. The eggs are 65 by 75 μ.

The tail of the male ends in a pointed tip. The spicules are 4·2–4·3 mm. long in the longest males. There are five pairs of single and one pair of double papillae on the tail; in front of these on each side lies a group of five papillae, appearing as adanal and just post-anal in young specimens, but all adanal in old males; eighteen pairs of pre-anal papillae are arranged in two parallel rows on each side; and anterior to these are sixteen pairs of papillae in a single row on each side.

The species differs from all *Contracaecum* spp. of which the description of the male is available here, with the exception of *C. scotti* Leiper & Atkinson 1914 (from *Diomedia melanophris*), in the presence of the double rows of papillae pre-anally. The present species from penguins differs from *Contracaecum scotti* in the shape of the oesophagus, in the length of the intestinal caecum, and in the more anterior position of the vulva. The shape of the interlabia and the form of the tail of the male are strikingly similar in the two species. *C. heardi* is distinguished from *C. eudyptes* (Johnston & Mawson 1952) taken from *Eudyptes* sp. from Campbell Island, by the proportions of the parts of the alimentary canal.



Figs. 1–5. *Contracaecum heardi* n.sp.; figs. 1 and 2 dorsal and ventral views of head; fig. 3, oesophageal region; fig. 4, tail of male 23 mm. long; fig. 5, tail of male 10·3 mm. long. Figs. 1 and 2 to same scale; figs. 4 and 5 to same scale.

Table 1

(The phocascarid larva is a pre-adult stage occurring in at least some *Contracaecum* spp. (see Johnston & Mawson, 1945) in which the larval tooth is absent and the lips well developed, but in which the interlabia are absent. *l*=body length; *o*=oesophagus; *oa*-oesophageal appendix; *ic*=intestinal caecum; *sp*=spicule.)

Stage	*l* (mm.)	*o* : *l*	*oa* : *o*	*ic* : *o*	*sp* : *l*
'*Phocascarid*'	3·4–3·6	1 : 4	1 : 3–4	1 : 2–1·6	–
Smallest males	8–10·3	1 : 5–8	1 : 4–3	1 : 1·6	1 : 2·6
Smallest females	6–10·7	1 : 5–8	1 : 3	1 : 1·3	–
Adult males	17–23	1 : 7–9	1 : 4	1 : 1·3–1·5	1 : 4–5
Adult females	40–48	1 : 12	1 : 4	1 : 1·3	–

The material examined for this report includes young and adult specimens. In some cases worms of different ages were taken from the same host individual so it is possible to compare the proportions of parts at different stages (Table 1). The relatively large numbers of this parasite found in penguins at Heard Island make it necessary to modify some of the suggestions made by Johnston & Mawson (1945) as to the identity of some nematodes recorded from penguins. Linstow (1907, p. 470) recorded *Ascaris* sp., 51 mm. long, from *Catarrhactes* (=*Eudyptes*) *chrysolophus*; it was

suggested in 1945 that this might have been a chance infection; it now seems probable that it was an adult female of *Contracaecum heardi*, which is now described from the same host species. *C. antarcticum* (Johnston), an immature form with larval tooth, has twice been recorded from *Pygoscelis adeliae* (Johnston, 1938; Johnston & Mawson, 1945), and in the latter paper was referred to *Contracaecum osculatum* as an accidental infection. It is an earlier stage larva than any found in *Pygoscelis papua*; the oesophageal appendix is strikingly longer in proportion to the oesophagus than in the youngest of *Contracaecum heardi*. In the same work (Johnston & Mawson, 1945, p. 121) a brief description was given of six immature female worms of a species of *Contracaecum* from *Aptenodytes forsteri*, and they also were identified as an accidental infection of *Contracaecum osculatum*; these worms differ from *C. heardi* in the same feature as does *C. antarcticum*, namely, in the longer oesophageal appendix, and also in the position of the vulva. It is possible that both are immature *C. osculatum* (accidental infection) as previously suggested, but they may be young forms of a species (*C. antarcticum* Johnston) of which the adult is still undescribed.

Paranisakiopsis sp.

An incomplete specimen, lacking the caudal end, was taken from *Phoebetria palpebrata*, HZ/49/246 (18), from Heard Island. The characters of the head and oesophagus are those of species of the genus *Paranisakiopsis*; it is probable that the specimen was ingested with the food, as this genus has so far been recorded only from fish.

Parafilaroides hydrurgae n.sp. (Figs. 6–9)

Two collections were made at Heard Island of worms from the respiratory tract of *Hydrurga leptonyx*, one, HZ/49/142 (140), from the lung, consisting of one female worm, and the other, HZ/49/128 (127), from the bronchi, consisting of three male and twenty female worms. The males are 25·0–36·7 mm. long, the females up to 90 mm.; the cuticle of most specimens is more or less loose and inflated, though this may be an artifact. There are six very small lips, and six labial papillae. The oseophagus is short and cylindrical, 0·18 mm. long in the male, up to 0·25 mm. in the female. In some specimens the ventral glands are distinct, extending to about 0·4 mm. from the head. The excretory pore is just behind the lips. On the tail of the male there is no sign of bursa or papillae in lateral view, though in the ventral view there are possibly three or perhaps five small papillae. The spicules, 55–60 μ long, have each a knob-like proximal end. The gubernaculum is 30 μ long, and simple in form. In the female the tail is rounded, the anus 30 μ from the tip, the vulva 60 μ in front of anus. The vagina vera is apparently 30 μ long; the united part of the uteri, or the vestibule, 0·45 mm. long. This and the doubled uteri are packed with coiled larvae, the largest of which is 0·31 mm. long, and 11 μ in maximum diameter. They have truncated anterior ends, and tails tapering quickly to an acicular point.

This species undoubtedly belongs to the genus *Parafilaroides* Dougherty 1946; the sphincter of the vulva, apparently of a transparent matrix, is present in all specimens. The uterine wall adjacent to it is apparently surrounded by several cells with large nuclei. The species is distinguished from others of the genus (see Dougherty, 1947) by its greater length, by the spicule length, by the length of the gubernaculum, and by the relation between these last two lengths.

Seuratia shipleyi (Stossich) (Fig. 10)

One female worm belonging to this species was taken from *Phoebetria palpebrata*, HZ/49/246 (18), from Heard Island. The worm is 23·5 mm. long, with the vulva 12 mm. from the head. The vestibule is 190 μ long, and the cervical papillae 150 μ from the lips. The oesophagus is 5·7 mm. long. The eggs are 25 by 15 μ. The specimen is referred to Stossich's species, as this is the only one recorded from the Southern hemisphere, but there is little to distinguish the female of *Seuratia shipleyi* (Stoss.) from that of *S. puffini* (Yamaguti) 1941.

Ascarophis nototheniae (Johnston & Mawson)

The nematode is apparently a common parasite of *Notothenia coriiceps* and *N. cyanobrancha* at Heard Island. It was present in the following fish: *N. cyanobrancha*, HZ/49/fishes 37, 161, 347, 614, 680, 688, and *N. coriiceps*, HZ/49/fishes 159, 421.

Stegophorus paradeliae (Johnston & Mawson) (Fig. 11)

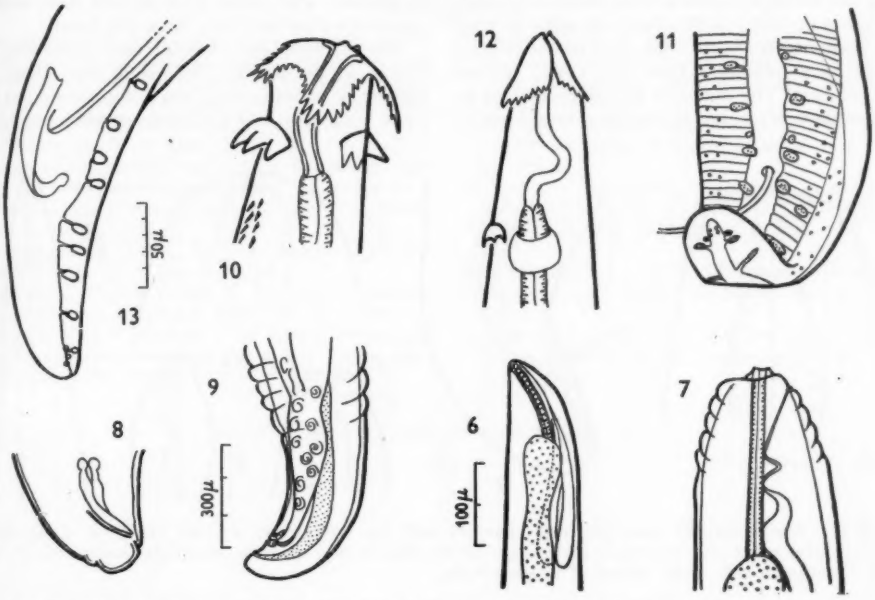
This nematode, described from one female individual from *Pygoscelis adeliae*, is now recorded from several penguins from Heard Island. The material consists of several males and females from *Pygoscelis papua* HZ/49/205 (145); one female from *Eudyptes cristatus*, HZ/49/445 (442), and one female from *Eudyptes chrysolophus*, HZ/49/629 (620). A more detailed account of the parasite can now be given.

The number of collar spines and the relative positions of cervical papillae, end of vestibule, and nerve cord, are as described for the species. In the present material, the central cusp of the cervical papillae is shorter than the other two in some cases.

The male is up to 9·2 mm. long. The cervical papillae are 0·23 mm. from the head, the vestibule 0·19 mm. long, the anterior and posterior parts of

the oesophagus are respectively 0·49 and 1·7 mm. long. Thick caudal alae extend about 1·2 mm. from the end of the tail; they are transversely striated and dotted with refractive points on their ventral surfaces. There are four pairs of pre-anal and six pairs of post-anal papillae (Fig. 11). The major spicule is 1·25 mm. long, needle-like, ending in a small crochet hook; the shorter is 0·09 mm. long, and spatulate in form.

The females are up to 17 mm. long; the cervical papillae are 0·26 mm. from the head; the vestibule (698), all from the stormy petrel, *Oceanites oceanicus*, and the anterior end of an adult ovigerous female, from *Pelecanoides georgicus*, HZ/49/Y509 (509). The young male (coll. 706) is taken as the type, and the juvenile and fragment are considered to belong to the same species. The length is 2·85 mm.; there are 40–44 spines on the collarette; the cervical papillae, 0·11 mm. behind the anterior end, are trifid, the central cusp of each distinctly shorter than the others. The vestibule is 0·13 mm. long, sinuous and relatively narrow, 7–8 μ in diameter throughout.



Figs. 6–9. *Parafilaroides hydrurgae* n.sp.; figs. 6 and 7, views of the anterior end; fig. 8, tail of male; fig. 9, tail of female. Fig. 10, head of *Seuratia shipleyi* (Stoss.). Fig. 11, *Stegophorus paradeliae* Johnston & Mawson, tail of male. Figs. 12–13, *S. heardi* n.sp.; fig. 12, anterior end; fig. 13, tail of male. Figs. 6, 10 and 11 to same scale; figs. 7 and 9 to same scale; figs. 8, 12 and 13 to same scale.

is 0·21 mm., the anterior oesophagus 0·77 mm., and the posterior oesophagus 2·4 mm., in length. The vulva is 5·1 mm. or 1/3·3 of the body length, from the posterior end. The eggs are 20 by 40 μ. In the original description the eggs were stated to be 38 μ wide, a misprint for 18 μ.

Stegophorus heardi n.sp. (Figs. 12, 13)

Several nematodes which appear to belong to a new species of the genus *Stegophorus* were taken from two species of petrels at Heard Island. Unfortunately all individuals are either broken or immature, or both. The material consists of one young male and one very small juvenile, HZ/49/706 (701), and the posterior end of a male, HZ/49/699

The anterior part of the oesophagus is 0·44 mm. and the posterior 0·75 mm. in length. The nerve cord surrounds the oesophagus immediately behind its junction with the vestibule.

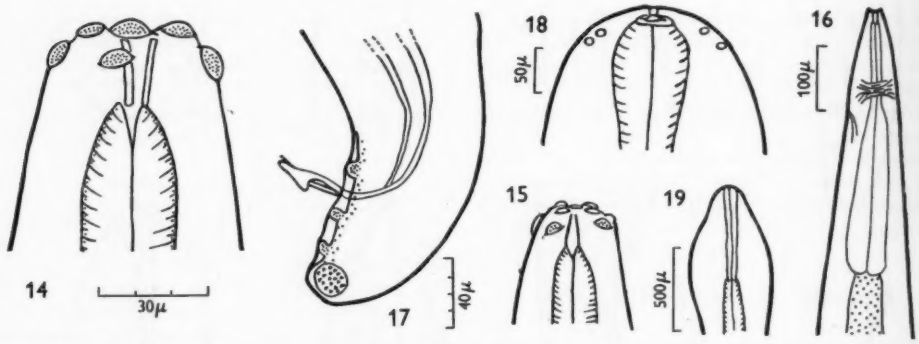
The longer spicule is 0·6 mm., the shorter 0·095 mm., in length. There are four pairs of pre-anal and eight pairs of post-anal papillae; the two pairs nearest the tip of the tail, non-pedunculate and very small, are seen most clearly in lateral view. In the male specimen, of which only the posterior part is present, the form of the tail and arrangement of papillae is similar but the length of the longer spicule is 0·92 mm., that of the shorter 0·09 mm.

In the female fragment, 5·9 mm. in length, the length of the vestibule is 0·13 mm., of the anterior

oesophagus 0·54 mm., of the posterior oesophagus 1·26 mm. The cervical papillae are relatively larger, and the three cusps are of equal length. The eggs are 44–45 μ by 22–23 μ. Though from a different host species, this fragment is assigned to *Stegophorus heardi* because of the similarity in structure of the collar, the sinuous form of the vestibule, and in the relation between the cervical papillae and posterior end of the vestibule.

The juvenile, or, perhaps more properly, larval, specimen, is 1·26 mm. long; the cervical papillae, minute and bicuspid, are 60 μ from the anterior end. The sinuous vestibule is 80 μ long; the anterior part of the oesophagus is 0·19 mm., the posterior part 0·43 mm. The excretory pore lies shortly behind the nerve ring. The collar is not festooned as in adult members of the genus, but lies straight across the body except for slight indentation dorsally and ventrally. Its posterior border is not toothed, but appears as if finely scalloped, these small folds being far more numerous than the forty teeth found in the adult collar.

The species is very close to *S. paradeliae*, but differs in the form of the vestibule which is always sinuous, whereas in all specimens of *S. paradeliae* which we have seen, it is straight. It differs also in the sizes of the spicules relative to one another, (1 : 14 in *S. paradeliae*, 1 : 6·3–10 in *S. heardi*). *S. heardi* differs from *S. pachyptilae* (Johnston & Mawson) in the size of the eggs, the position of the cervical papillae, and in the number of collar serrations.



Figs. 14–17. *Desmidocercella australis* n.sp.; figs. 14 and 15, lateral and ventral views of head; fig. 16, oesophageal region; fig. 17, tail of male. Figs. 18–19, *Filaria* (s.l.) sp. from *Mirounga leonina*; fig. 18, head; fig. 19, anterior end. Figs. 15 and 17 to same scale.

Desmidocercella australis n.sp. (Figs. 14–17)

Two small male worms were taken from the trachea and bronchus of *Phalacrocorax atriceps nivalis*, HZ/49/200 (162). The worms are 3·9–4·0 mm. in length. The cuticle is finely striated, with rows of punctations between the striae. The mouth is surrounded by eight large sub-median papillae arranged in two circles. The buccal capsule, with walls about 2 μ thick, is about 20 μ long, its dorsal and ventral walls about 8 μ apart, its lateral walls converging towards the mouth. The oesophagus is 0·4 mm. long; the two parts are not morphologically distinguishable except that posterior to the nerve ring the oesophageal diameter is greater. The nerve ring is 0·16 mm. and the excretory pore 0·18 mm. from the head.

The spicules are unequal and dissimilar. The longer is needle-like, 0·55–0·62 mm. long, the shorter, 0·24 mm. long, has a slipper-shaped end-piece. Narrow caudal alae are present, supporting three pairs of papillae of which one is pre-anal. Subterminally placed on each side of the tail is a circular cushion-like area beset with small rather pointed tubercles.

This species is close to *Desmidocercella incognita* Ssolonitzin (1932). In view of the presence of caudal alae and the absence of a fourth pair of caudal papillae, as well as the small size of the buccal capsule, a new species, *D. australis*, is proposed for the Heard Island specimens.

Filaria (s.l.) sp. (Figs. 18, 19)

Part of a filarial worm was taken from a blood vessel of *Mirounga leonina*, HZ/49/306 (303). The fragment is the anterior end of a female worm, and is 195 mm. long, with a maximum diameter of 0·57 mm.

The cuticle of the head is thickened, though not forming 'epaulettes'. There are five pairs of

cephalic papillae. The mouth is small, leading to a short cylindrical vestibule, the base of which is surrounded by a cuticular ring. The anterior part of the oesophagus is 0·6 mm. long, the posterior 3 mm.; the nerve ring lies 0·4 mm. from the mouth. The body is distinctly swollen at the level of the junction of the two parts of the oesophagus, giving the anterior end a fusiform appearance. The uteri are filled with small coiled embryos. The vulva was not seen.

Capillaria convoluta (Fourment)

Several specimens of this species belonging to both sexes were taken from *Macronectes giganteus*, HZ/49/613 (611), from Heard Island. They are somewhat stouter than those described by Johnston & Mawson (1945, 151) from *Macronectes giganteus* and *Thalassarche melanophris*. They agree, however, in the spicule length, the presence of a spinous spicule sheath, and in the shape and size of the eggs.

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